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PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **304th Meeting** *of the Biochemical Society was held in the Middlesex Hospital Medical School, London, W. 1, on Saturday, 19 January 1952, when the following papers were read:*

COMMUNICATIONS

Ribonucleic Acid and Deoxyribonucleic Acid during Tissue Regeneration. By D. H. Marrian (introduced by Barbara E. Holmes). (*Department of Radiotherapeutics, University of Cambridge, and the Sloan-Kettering Institute for Cancer Research, New York City*)

It is still an open question whether, during tissue growth, deoxyribonucleic acid can be synthesized at the expense of ribonucleic acid. Some investigations by Brachet (1933) on the development of sea urchin eggs supported this hypothesis, but further observations since then have been confusing. Thus, the newer analytical techniques developed by Schmidt, Hecht & Thannhauser (1948) disproved the experimental foundations for Brachet's suggestion, and Villee, Lowen, Gordon, Leonard & Rich (1949) showed that it was unlikely that ribonucleic acid phosphorus was a precursor of deoxyribonucleic acid phosphorus. Abrams (1951) using ^{14}C labelling in the same organism came to similar conclusions with regard to the carbon skeleton. However, the well known mitotic inhibition caused by small doses of X-radiation (Henshaw & Cohen, 1940) was considered by Mitchell (1940, 1942) as due to the inhibition of the synthesis of deoxyribonucleic acid, and the simultaneous increase in cytoplasmic ultraviolet absorption was possibly due to an accumulation of ribonucleic acid which normally would have been used for deoxyribonucleic acid synthesis. To further our knowledge of tissue growth, it was obviously of interest to show whether deoxyribonucleic acid could be synthesized from ribonucleic acid in a different biological system.

The initial observation by Brown, Roll, Plentl & Cavalieri (1948) that adenine could be utilized for nucleic acid synthesis was followed by the demonstration that the incorporation was much greater into ribonucleic acid than into deoxyribonucleic acid of resting tissue (Furst, Roll & Brown, 1950). This is the basis of the experiments here presented. Adenine labelled with ^{14}C was given to a group of rats over 3 days. Twenty-four hours following the final injection, partial hepatectomy was performed, and the liver allowed to regenerate for 48 hr. The animals were then killed and the regenerated liver removed. Both sets of livers were investigated, and the amounts of radioactivity residing in the separate purines of the ribo- and deoxyribo-nucleic acids were compared. Initial experiments have shown that during liver regeneration the activity of the ribonucleic acid purines fell, while that of the deoxyribonucleic acid purines rose, and amplifications of this type of experiment, now in progress, can be expected to show whether this represents true deoxyribonucleic acid synthesis from ribonucleic acid in this biological system.

REFERENCES

Abrams, R. (1951). *Exp. Cell Res.* **2**, 235.
Brachet, J. (1933). *Arch. Biol., Paris*, **44**, 519.
Brown, G. B., Roll, P. M., Plentl, A. A. & Cavalieri, L. F. (1948). *J. biol. Chem.* **172**, 469.
Furst, S. S., Roll, P. M. & Brown, G. B. (1950). *J. biol. Chem.* **183**, 251.
Henshaw, P. S. & Cohen, I. (1940). *Amer. J. Roentgenol.* **43**, 917.
Mitchell, J. S. (1940). *Nature, Lond.*, **146**, 272.
Mitchell, J. S. (1942). *Brit. J. exp. Path.* **23**, 296.
Schmidt, G., Hecht, L. & Thannhauser, S. J. (1948). *J. gen. Physiol.* **31**, 203.
Villee, C. A., Lowen, S. M., Gordon, M., Leonard, E. & Rich, A. (1949). *J. cell. comp. Physiol.* **33**, 93.

Assay of Arylsulphatase of Rat Tissues. By K. S. Dodgson, B. Spencer and J. Thomas. (*Physiology Institute, Newport Road, Cardiff*)

Available colorimetric and spectrophotometric methods of determining arylsulphatase activity are based on the estimation of phenol (Abbott, 1947), *p*-nitrophenol (Huggins & Smith, 1947), 4-nitrocatechol (Robinson, Smith & Williams, 1951), *p*-chlorophenol or *p*-hydroxybenzaldehyde (Robinson, Spencer & Williams, 1951) liberated from the respective potassium sulphate esters.

Disadvantages in the use of potassium phenylsulphate as a substrate for arylsulphatase assay are, in comparison with other methods, the inconvenience of the procedure and the insensitivity and unspecificity of the final colour reaction. It is perhaps significant that the originator of the method has, in a later method, used *p*-nitrophenylsulphate (Abbott & East, 1949). The spectrophotometric method using potassium *p*-chlorophenylsulphate is also unsuitable for rat tissues since at 245 mμ., the wavelength of maximum absorption of *p*-chlorophenol, the interference by protein, even after 'deproteinization', is considerable.

We have found that attempts at simple fractionation of rat-tissue homogenates by centrifugation and subsequent use of supernatant solutions, as described by Huggins & Smith (1947), lead to large losses of arylsulphatase. We have found this to be due to the retention of the enzyme in the residues and, for this reason, the assay methods mentioned above have now been investigated with a view to their application to crude rat-tissue homogenates. Added *p*-nitrophenol, 4-nitrocatechol and *p*-hydroxybenzaldehyde can be quantitatively recovered from homogenates in acetate buffers before incubation but, after incubation with rat-liver preparations, significant losses of these phenols occur. Spectrophotometric evidence suggests that *p*-nitrophenol is reduced to *p*-aminophenol whilst *p*-hydroxybenzaldehyde is oxidized to *p*-hydroxybenzoic acid.

A search has therefore been made for phenols which can be recovered quantitatively from rat-tissue homogenates and which are also suitable for assay purposes. These requirements are filled by *p*-hydroxyacetophenone, $\lambda_{max.}$ 327·5 mμ., $\epsilon_{max.}$ 21,700 in 0·1N-NaOH; potassium *p*-acetylphenylsulphate has negligible absorption at this wavelength. The spectrophotometric method of estimation using this substrate is similar to that described for β-glucuronidase using *p*-chlorophenylglucuronide (Spencer & Williams, 1951). Using potassium *p*-acetylphenylsulphate, the arylsulphatase of rat liver is found to be optimally active at a substrate concentration of 0·007M and at pH 7·2 in 0·5M-acetate buffer. Defining a *p*-hydroxyacetophenone unit of arylsulphatase activity as that which in 1 hr. liberates 1 μg. of *p*-hydroxyacetophenone from 0·007M-substrate in 0·5M-acetate buffer, pH 7·2, at 37·5°, the activities of various rat tissues were: liver, 1450–2400; kidney, 420–650; spleen, 380–540; intestine, 70–110; adrenals, 550–810 units/g. of wet tissue.

REFERENCES

Abbott, L. D. (1947). *Arch. Biochem.* **15**, 205.
Abbott, L. D. & East, M. K. (1949). *Fed. Proc.* **8**, 178.
Huggins, C. & Smith, D. R. (1947). *J. biol. Chem.* **170**, 391.
Robinson, D., Smith, J. N. & Williams, R. T. (1951). *Biochem. J.* **49**, lxxiv.
Robinson, D., Spencer, B. & Williams, R. T. (1951). *Biochem. J.* **48**, xxviii.
Spencer, B. & Williams, R. T. (1951). *Biochem. J.* **48**, 537.

Tryptophan Dipeptides in Bacterial Nutrition. By J. H. Marshall and D. D. Woods. (*Department of Biochemistry, University of Oxford*)

The use of anti-metabolite inhibitors as tools in biochemical investigations has been applied to a study of the utilization of amino-acids, using amino-acid analogues as growth inhibitors.

4-Methyltryptophan was synthesized essentially according to Rydon (1948). Its ability to inhibit the growth of *Bacterium typhosum* has been described by Fildes & Rydon (1947). It has now been shown to inhibit the growth of *Streptococcus faecalis* R, *Leuconostoc mesenteroides* P60, *Lactobacillus arabinosus* 17–5, two strains of *Staphylococcus aureus* (one requiring tryptophan, the other not), and a strain of *Escherichia coli*. In each case competition between tryptophan and 4-methyltryptophan has been shown.

Of the amino-acids, tryptophan is the most effective antagonist of 4-methyltryptophan inhibition, but tyrosine and phenylalanine also show some activity, especially in the presence of tryptophan. Dittmer, Ellis, McKennis & du Vigneaud (1946) found that inhibition of *Esch. coli* by β-2-thienylalanine is overcome by tyrosine or phenylalanine, and also less effectively by tryptophan. We have confirmed these results with our strain of *Esch. coli* and with *Staphylococcus aureus*. Further evidence for a close connexion between the mode of action of these two inhibitors is provided by lack of synergism, and by the fact that strains of *Esch. coli* rendered resistant to 4-methyltryptophan also develop resistance to thienylalanine.

It is suggested that tyrosine and phenylalanine are involved in the utilization of tryptophan; to test this hypothesis the synthesis of the four dipeptides containing tryptophan and tyrosine or

phenylalanine was undertaken. The carbobenzyloxy method was adopted in each case with coupling via the azide.

As growth factors for the tryptophan-requiring organisms, none of the dipeptides was more active than tryptophan itself. For all the organisms tested except *Esch. coli*, however, the dipeptides were more active than tryptophan in overcoming 4-methyltryptophan inhibition. For *Strept. faecalis* R and *Staph. aureus*, L-tyrosyl-L-tryptophan and L-tryptophyl-L-phenylalanine overcame the inhibition non-competitively and at growth factor level.

The results indicate that one or more of these dipeptides may lie on the normal metabolic pathway by which tryptophan is utilized.

REFERENCES

Dittmer, K., Ellis, G., McKennis, H. jun. & du Vigneaud, V. (1946). *J biol. Chem.* **164**, 761.
Fildes, P. & Rydon, H. N. (1947). *Brit. J. exp. Path.* **28**, 211.
Rydon, H. N. (1948). *J. chem. Soc.* p. 705.

An Inhibitory Substance in the Gut of Chickens. By N. J. Berridge and J. Barrett. (*National Institute for Research in Dairying, University of Reading*)

The gut contents of normal chicks were found to be inhibitory to *Streptococcus agalactiae* in turbidimetric assays with incubation times of 90 min. Contents removed from the duodenum by squeezing, thus containing an unknown proportion of mucosa, were even more inhibitory, being approximately equivalent to a solution of 70 μg. of penicillin/ml.

The substance was extracted from the tissue by stirring with distilled water at the natural pH of 6–7, but the extract remained opalescent even after centrifuging for 20 min. at 15,000 rev./min. The inhibitory power of the extract was unimpaired after 24 hr. at room temperature at pH values 1–4 and 10, and was associated with a precipitate which reached a maximum at pH 3.

When an assay by the cylinder plate method was attempted, and diffusion was allowed to go on overnight at 10°, the clear zones were limited to the agar directly beneath the cups. A very low rate of diffusion was also found on dialysis in cellophan tubing.

We wish to thank Dr M. E. Coates for supplying gut contents from chickens reared on various diets.

Partition of Phosphorus in Relation to Phosphatase in Colostrum and Milk of Cows. By R. Chanda and E. C. Owen. (*The Hannah Dairy Research Institute, Kirkhill, Ayr*)

In recent experiments at this Institute it was shown that when lactating cows were treated with thiouracil there was a dramatic increase in the concentration of phosphatase in their milk, while at the same time there was a big decrease in ester and lipid phosphorus (Chanda & Owen, 1951). Casein phosphorus also showed a transient decrease. All these fractions were closely but negatively correlated with the enzyme. Thyroxine caused an equally marked but reverse effect, so that the large negative correlation persisted. The same correlations were also observed in human colostrum and transitional milk (Chanda, Owen & Cramond, 1951) and in goats' milk (Chanda, McNaught & Owen, 1950), in which the phosphatase titres are naturally much smaller than in cows' milk.

In view of these findings, the natural variations in the partition of phosphorus in cow colostrum and milk were also studied so as to ascertain whether the above-mentioned correlations applied to them. Observations were also made on the change in the ratio of N:P during the transition of colostrum to milk and with advancing lactation. The results showed that both the nitrogen and phosphorus contents of the initial post-partum secretions were large and the ratio of N:P was 11:1. During the first week both the nitrogen and phosphorus content rapidly decreased, but the decrease in nitrogen was greater than that of phosphorus so that the ratio of N:P dropped to a value of 4·5:1 at the end of the week. Thereafter the ratio increased throughout lactation attaining a value of 7·5:1 at the 40th week.

When the inorganic, ester, lipid and casein phosphorus were expressed as percentages of the total P it was found that the percentage of inorganic P was 60 at parturition, falling to 50 on the sixth day, at which value it remained until the fourth week post-partum. A similar trend was observed in the phosphatase which was 167 phenol units

initially and 14 units on the sixth day. Neither the phosphatase nor the inorganic P had changed much by the fourth week. By the sixteenth week, however, inorganic P was 63% of the total P and the phosphatase titre was 125. By the 40th week these figures had become 72% and 195 units, respectively. Definite trends were shown in the ester and lipid P figures, but they were the reverse of trends shown by inorganic P and phosphatase. Thus phosphatase was correlated positively with inorganic P and negatively with ester and lipid P. The casein P figures were less easy to interpret, being initially larger and finally smaller than their own average. Initially ester, lipid and casein P were 9·3, 11·3 and 19% of the total P, respectively. At the fourth week these figures had become 14·0, 14·2 and 18%, respectively. At the 40th week they were 4·2, 9·9 and 14%, respectively.

REFERENCES

Chanda, R., McNaught, M. L. & Owen, E. C. (1950). Unpublished observations.
Chanda, R. & Owen, E. C. (1951). *Biochem. J.* **50**, 100.
Chanda, R., Owen, E. C. & Cramond, B. (1951). *Brit. J. Nutrit.* **5**, 228.

β-Carotene in Goat Liver and Colostrum. By R. CHANDA and E. C. OWEN. (*The Hannah Dairy Research Institute, Kirkhill, Ayr*)

In experiments with lactating goats it was noted that the colostrum of the animals varied from orange to yellow. In view of the well known extreme whiteness of goat milk and of the absence of carotenoids from it, the pigment of the colostrum was examined spectrophotometrically after having been chromatographically purified on alumina as described by Chanda, Owen & Cramond (1951). It was found that the yellow pigment contained β-carotene accompanied by another yellow lipochrome which was assumed to be lutein, but was not measured in the spectrophotometer. The pigment which occupied the β-carotene position in the column could be eluted with 3% acetone in *n*-hexane. That this pigment was β-carotene was demonstrated by comparing its properties with pure β-carotene (British Drug Houses Ltd.) itself purified in the laboratory by chromatography. The pigments from both sources when mixed and chromatographed gave only a single band. Their absorption spectra in *n*-hexane solution between 350 and 500 mμ. demonstrated that the maxima of absorption for the pigment from goat colostrum and β-carotene were 423, 451 and 475 mμ. These maxima are concordant with those obtained in *n*-hexane by Karrer & Jucker (1950) and with the values obtained in light petroleum by Morton (1942).

The amount of β-carotene varied from 12 to 46 μg./100 ml. of colostrum in the six goats examined. It was thought that the β-carotene in the colostrum probably came from the liver. Accordingly, the livers of the same six goats were examined *post mortem* for β-carotene. The result showed that β-carotene was present in most of the livers, but the amount was extremely variable (0–0·84 μg./g. liver). It was of the order which would be expected if the amount secreted in the colostrum were determined by the amount present in the liver. The chromatographic and spectrophotometric properties of this liver lipochrome were the same as those of colostral β-carotene. The kidneys of the goats differed from the livers in not containing any lipochromes.

The livers of four of the goats examined by us resembled those of the ewes investigated by Ganguly & Deuel (1951) which contained both carotene and lutein. The livers of the remaining two goats resembled those of the goats examined by Goodwin & Gregory (1948) from which β-carotene was absent.

REFERENCES

Chanda, R., Owen, E. C. & Cramond, B. (1951). *Brit. J. Nutrit.* **5**, 228.
Ganguly, J. & Deuel, H. J. (1951). *Fed. Proc.* **10**, 383.
Goodwin, T. W. & Gregory, R. A. (1948). *Biochem. J.* **43**, 505.
Karrer, P. & Jucker, E. (1950). *Carotenoids.* Translation by E. A. Braude. Amsterdam: Elsevier.
Morton, R. A. (1942). *The Application of Absorption Spectra to the Study of Vitamins, Hormones and Coenzymes*, 2nd ed. London: Adam Hilger.

The Synthesis of Methionine from Homocysteine by *Escherichia coli*. By F. Gibson and D. D. Woods. (*Department of Biochemistry, University of Oxford*)

The evidence that vitamin B_{12} and *p*-aminobenzoic acid (or folic acid) are concerned in the synthesis of methionine from homocysteine in animals (e.g. Oginsky, 1950; Bennett, 1950) and in micro-organisms (e.g. Davis & Mingioli, 1950; Strehler, 1950), although considerable, is indirect. Using suspensions, an attempt has been made to provide more direct evidence that these two vitamins are involved in the bacterial synthesis of methionine.

Cells deficient in *p*-aminobenzoic acid were obtained by growing a mutant strain of *Escherichia coli* requiring *p*-aminobenzoic acid for growth (Lampen, Jones & Roepke, 1949) in an enriched medium with minimal *p*-aminobenzoic acid. Such cell suspensions in phosphate buffer, incubated with glucose and homocysteine, failed to synthesize methionine, as estimated by microbiological assay with *Leuconostoc mesenteroides* P 60, unless *p*-aminobenzoic acid was added. Synthesis occurred on addition of 10^{-8}M-*p*-aminobenzoic acid and became optimal at 10^{-7}M. All the components of the system were essential.

Suspensions of B_{12}-deficient cells were obtained by growing a mutant of *Esch. coli* requiring B_{12} or methionine for growth (Davis & Mingioli, 1950) on a methionine medium. Methionine synthesis (approx. 0·1 μmol./mg. dry cells/hr.) depended on the addition of 0·1–1·0 μmg./ml. of vitamin B_{12} to a system similar to that described above. Addition of B_{12} also increased synthesis (3- to 9-fold) by other strains of *Esch. coli* and a strain of *Proteus vulgaris*, none of which required added B_{12} for growth.

In these systems there was no added methyl donor, and to gain more information as to the action of the vitamins an attempt was made to find the source of the methyl group. Synthesis was inhibited 70–100% by 20% CO_2; this inhibition was overcome by serine (10^{-2}M), but not by choline, glycine, formate or acetate (10^{-2}M). Synthesis was somewhat stimulated by serine. Experiments with a mutant requiring either serine or glycine for growth showed that either of these amino-acids was essential for synthesis. As synthesis proceeds more rapidly with serine, it was concluded that serine is the intermediate more directly concerned.

A possibility, at present under investigation, is that methionine formation under the experimental conditions occurs by the condensation of homocysteine and serine to give cystathionine which then undergoes direct cleavage; this has also been suggested recently by Block, Stekol & Loosli (1951).

REFERENCES

Bennett, M. A. (1950). *J. biol. Chem.* **187**, 751.
Block, R. J., Stekol, J. A. & Loosli, J. K. (1951). *Arch. Biochem.* **33**, 353.
Davis, B. D. & Mingioli, E. (1950). *J. Bact.* **60**, 17.
Lampen, J. O., Jones, M. J. & Roepke, R. R. (1949). *J. biol. Chem.* **180**, 423.
Oginsky, E. L. (1950). *Arch. Biochem.* **26**, 327.
Strehler, B. L. (1950). *J. Bact.* **59**, 105.

Some Properties of Vitamin B_{12}-like Factors from Calf Faeces. 1. Characteristics of Different Fractions. By J. E. Ford and J. W. G. Porter. (*National Institute for Research in Dairying, University of Reading*)

We have shown (Ford, Kon & Porter, 1951) that the vitamin B_{12} activity of calf gut contents and faeces can be separated into three main fractions: *A*, *B* and vitamin B_{12}. We have now found that paper chromatography reveals a fourth fraction, *C*, slower-moving than fraction *A*. *C* is about 15 times more active for *Bacterium coli* (*Escherichia coli*) than for *Lactobacillus leichmannii*, and is slightly active for *Euglena gracilis*.

The vitamin B_{12} activities of purified extracts of calf faeces were separated by partition chromatography on damp kieselguhr. Development with water-saturated *sec.*-butanol containing a trace of cyanide gave two pink bands: the lower, containing fraction *B* and vitamin B_{12}, the higher, fraction *A*.

Further purification by chromatography of fraction *A* gave a red substance that crystallized from aqueous acetone in clumps of needles. Its absorption spectrum closely resembles that of vitamin B_{12}, with peaks at 280, 320, 361 ($E^{1\%}_{1\,cm.}$ 116), 520 and 548 ($E^{1\%}_{1\,cm.}$ 32) mμ. Excess cyanide converts it to a purplish substance with peaks at 278, 305, 368, 540 and 578 mμ. The only noteworthy difference from the vitamin B_{12} spectrum is the absence of an inflexion at 288 mμ.

Passage of nitrogen through a warm aqueous solution of the crystalline material at pH 5 in strong light (Boxer & Rickards, 1951) causes the evolution of cyanide, and the formation of, presumably, an hydroxy compound, showing peaks at 274, 351

and 520 mμ. Addition of cyanide to it changes the absorption spectrum back to that of fraction *A*.

The microbiological potency of the crystalline material for *Bact. coli* and for *Euglena gracilis* (kindly assayed by Dr G. I. M. Ross) is almost identical with that of vitamin B_{12}, but only about one-fifth of it with *Lactobacillus leichmannii*.

Fraction *B* and vitamin B_{12} were separated on damp kieselguhr by development with wet *n*-butanol. The small amount of fraction *B* present and its apparent instability have, so far, prevented its complete purification. The purest preparation available has absorption peaks at 354 and 465 mμ. and is converted by the addition of excess cyanide to a purple compound with peaks at 348, 367, 530 and 580 mμ.

Fraction *B* is active for *Bact. coli* but almost inactive for *Lactobacillus leichmannii* and *Euglena gracilis*.

REFERENCES

Boxer, G. E. & Rickards, J. C. (1951). **30**, 382.
Ford, J. E., Kon, S. K. & Porter, J. W. G. (1951). *Biochem. J.* **50**, ix.

Some Properties of Vitamin B_{12}-like Factors from Calf Faeces. 2. Biological Activities and Interrelationships. By M. E. Coates, J. E. Ford, G. F. Harrison, S. K. Kon and J. W. G. Porter. (*National Institute for Research in Dairying, University of Reading*)

Coates, Ford, Harrison, Kon, Porter, Cuthbertson & Pegler (1951) showed that chick assays of vitamin B_{12} in gut contents and faeces of calves give lower results than do microbiological measurements. Chick tests of the fractions isolated by Ford & Porter (1952) showed that fraction *A* given by mouth had no vitamin B_{12} activity at a level of 20 μg./100 g. diet (about 100 times the detectable level of vitamin B_{12}) and antagonized about one-fifth its own weight of vitamin B_{12}. Fraction *B** by itself was inactive at a dose of 20 μg./100 g. diet but increased the chicks' response to crystalline vitamin B_{12}. By intramuscular injection both fractions had an activity of the order of one-tenth that of injected crystalline vitamin B_{12}. A test kindly carried out by Dr J. N. Marshall Chalmers on one patient indicated that the anti-pernicious anaemia activity of fraction *A* was little short of that of vitamin B_{12}.

* We had not enough fraction *B* itself but a fermentation product kindly supplied by Dr E. Lester Smith and believed to be identical with fraction *B* because of its similar chromatographic, spectrographic and microbiological behaviour was used instead.

The biological properties of the fractions are reflected in the results of chick assays of whole faeces. Their apparent vitamin B_{12} content increased markedly when crystalline vitamin B_{12} was included in the test diets, possibly because the enhancement of the added vitamin by fraction *B* was greater than the antagonistic effect of fraction *A*.

Incubation of another sample of faeces at 37° for 72 hr. increased the vitamin B_{12} content for chicks from 0·3 to 0·6 μg./g. Microbiological assays of the material, in conjunction with bioautographs, showed marked increases in vitamin B_{12} and decreases in fractions *A*, *B* and *C*. The sum of the *Lactobacillus leichmannii* activities of the four fractions was the same before and after incubation, but the *Bacterium coli* (*Escherichia coli*) activity diminished (cf. Ford & Porter, 1952).

Usually some 70 % of the vitamin B_{12} activity of calf faeces for *Bact. coli* is contributed by fraction *A*, with fractions *B* and *C* and vitamin B_{12} about equally responsible for the rest. The changes on incubation in biological and microbiological potencies are compatible with this distribution.

REFERENCES

Coates, M. E., Ford, J. E., Harrison, G. F., Kon, S. K., Porter, J. W. G., Cuthbertson, W. F. J. & Pegler, H. F. (1951). *Biochem. J.* **49**, lxvii.

Ford, J. E. & Porter, J. W. G. (1952). *Biochem. J.* **51**, v.

Energy Intake and Ribonucleic Acid Metabolism. By H. N. Munro, D. J. Naismith and T. W. Wikramanayake. (*Department of Biochemistry, The University, Glasgow*)

The influence of energy intake on the quantity and rate of turnover of liver ribonucleic acid has been studied on rats. Experiments were carried out both at adequate levels of protein intake and with protein-free diets. Differences in energy intake were produced by varying the amount of carbohydrate. The rats were maintained for 4 days at a given energy intake before killing. Ribonucleic acid was separated from the liver by the Schmidt-Thannhauser technique and determined by P estimation. In the turnover experiments, ^{32}P was injected in the form of orthophosphate and the nucleotides subsequently isolated by ionophoresis (Smellie & Davidson, 1951). The specific activities of the nucleotides were then related to the activity of the liver inorganic phosphate (relative specific activity).

When the diet contained abundant protein, differences in energy intake had a considerable influence on the amount of ribonucleic acid in the liver. Taking as a reference standard the quantity present at an energy intake of 1200 kg. cal./sq.m. body surface area, it was observed that a change in energy intake of 1000 kg. cal./sq.m. caused an alteration of 31% in the amount of ribonucleic acid per liver. In the case of rats receiving the protein-free diet, the same change in energy intake resulted in an alteration of only 11% in the amount of ribonucleic acid. The rate of incorporation of ^{32}P was affected in the opposite way. When the diet provided adquate amounts of protein, the rate was not appreciably altered by the level of energy intake, whereas on the protein-free diet a change in energy intake of 1000 kg. cal./sq.m. caused the relative specific activity to increase by approximately 30% of the turnover rate at 1200 kg. cal.

Thus on high protein diets an increase in energy intake caused a rise in the total amount of ribonucleic acid per liver without appreciably affecting the percentage of P atoms exchanged in a given time, whereas on the protein-free diet it produced a minimal change in total ribonucleic acid but a considerable increase in the percentage of P atoms exchanged. This implies that the total number of P atoms incorporated in a given time into liver ribonucleic acid is related to energy intake and not to protein intake.

REFERENCE

Smellie, R. M. S. & Davidson, J. N. (1951). *Biochem. J.* **49**, xv.

The elimination of 4:6-Dinitro-*o*-cresol by Man and by Animals. By E. King and D. G. Harvey. (*Department for Research in Industrial Medicine, Medical Research Council Laboratories, London, N.W. 3, and the London Hospital, London, E. 1*)

In a recent communication, Smith, Smithies & Williams (1951) have demonstrated that rabbits excrete in urine less than 20% of a single dose of 4:6-dinitro-*o*-cresol (DNOC), as its metabolites, within 48 hr.

Barnes, Denz & Parker (1951) have also shown that repeated daily doses of DNOC do not accumulate in the rabbit. Further comparative studies, which will be the subject of fuller communications elsewhere, suggest that the efficiency in eliminating DNOC is in the order: rabbit > rat > man. Studies on the accumulation of DNOC in the blood of man (Harvey, Bidstrup & Bonnell, 1951) indicate that man can accumulate DNOC, giving blood levels of 15–20 μg./g., as the result of small (about 1 mg./kg.) daily doses. On the other hand, the rat can only attain a comparable level following much higher doses (10 mg./kg. by stomach tube give 20–25 μg./g.). The most efficient of the three, the rabbit, can only maintain a level of about 5 μg./g. on repeated daily doses as high as 25 mg./kg. (cf. Barnes *et al.* 1951). Blood DNOC levels fall off in an exponential manner whether they result from single or repeated daily doses given by stomach tube. Thus, for six female rabbits, 1×30 mg./kg., the slope of the curve $b = -0{\cdot}0448$; six male rabbits, 9×5 mg./kg., $b = -0{\cdot}0454$; five female rats, 1×30 mg./kg., $b = -0{\cdot}01123$; four male rats, 9×20 mg./kg., $b = -0{\cdot}01055$. There is no significant difference in the blood DNOC decay slopes of those animals receiving single doses from those receiving several daily doses. The study of the decay in blood levels of a reported clinical case (Pollard & Filbee, 1951) shows that for man $b = -1{\cdot}1280$. The half-times of elimination of DNOC from the blood of the rabbit, rat and man are 6·7 (6·6), 26·8 (28·5), and 153·6 hr. respectively. The figures in brackets are the half-times of elimination resulting from single doses.

Urinary excretion of DNOC gives further evidence of species difference in behaviour towards DNOC. Man excretes about 0·3% of the intake (about

1 mg./kg.) within 5 hr. of dosing and about 0·8 % within 24 hr. The rabbit, after a dose of 25 mg./kg., excretes 4–8 % within 5 hr. and 5–9 % within 24 hr. Although excretion of urinary DNOC ceases in the rabbit about 3 days after dosing, man continues to excrete DNOC 4–5 weeks later, when quantities of about 1 mg. may still be present in 24-hr. urine samples (Harvey *et al.* 1951).

REFERENCES

Barnes, J. M., Denz, F. A. & Parker, V. H. (1951). *Brit. J. industr. Med.* **8**, 226.

Harvey, D. G., Bidstrup, P. L. & Bonnell, J. A. L. (1951). *Brit. med. J.* **2**, 13.

Pollard, A. B. & Filbee, A. F. (1951). *Lancet*, **2**, 618.

Smith, J. N., Smithies, R. H. & Williams, R. T. (1951). Proc. 303rd Meeting Biochem. Soc.

Gas-Liquid Partition Chromatography. The Separation and Identification of Volatile Bases: (*a*) Aliphatic Amines, (*b*) Pyridine Homologues. By A. T. James. (*National Institute for Medical Research, Mill Hill, London, N.W.* 7)

Two papers (James & Martin, 1952; James, Martin & Howard Smith, 1952) to be published soon, will describe the separation of volatile fatty acids from formic acid to undecanoic acid and also of ammonia and mono-, di- and tri-methylamine by gas-liquid partition chromatography. This study has now been extended to include the higher aliphatic amines and pyridine homologues.

Aliphatic amines. By the use of columns having liquid paraffin as the stationary phase at a temperature of 100°, methylamine, ethylamine, *iso*propylamine, *n*-propylamine, *iso*butylamine, *n*-butylamine, di*iso*propylamine, *iso*amylamine, *n*-amylamine, di-*n*-propylamine, *iso*hexylamine, *n*-hexylamine, di-*sec.*-butylamine, *n*-heptylamine and di-*n*-butylamine may readily be separated in amounts lying between 0·3 and 10 μ-equiv. A few amines show incomplete separation, but where overlaps of primary, secondary and tertiary amines occur, the separation can be carried out by recourse to a column having 'Lubrol MO' (a polyethylene-oxide-fatty alcohol condensate) as a liquid phase. With such a polar phase, differences in H-bonding power (primary amines > secondary amines > tertiary amines) exist that exert such an effect on the retention volumes that ready separations of those primary, secondary and tertiary amine mixtures not separable on paraffin columns are possible.

A straight line relationship exists between the log relative retention volumes

$$\left(\frac{\text{Retention volume of amine}}{\text{Retention volume of ethylamine}}\right)$$

and the number of carbon atoms in the amines for each homologous series on each type of column. In this way the retention volume of any amine can be predicted from a knowledge of the retention volume of any other amine in the correct homologous series. For any homologous series of amines a straight-line relationship exists between the relative retention volumes on liquid paraffin columns and the relative retention volumes on 'Lubrol MO' columns. The slope of the lines varies with the homologous series; the order of line slopes is tertiary amines > secondary amines > primary amines. By the determination of the relative retention volumes on the two types of column it is possible to state whether an unknown amine is primary, secondary or tertiary. Of twenty-five different amines, only two pairs of amines, *sec.*- and *iso*butylamine, di-*sec.*- and di*iso*butylamine cannot be separated on one or other of the 4 ft. columns.

Pyridine homologues. By the use of columns having liquid paraffin as the stationary phase pyridine, α-picoline, β-picoline, 2:6-lutidine, 2:4-lutidine and 2:4:6-trimethylpyridine may be readily separated. The commercial samples of lutidine and collidine used in paper chromatography may be analysed in this way and some progress can be made in the standardization of such samples to prevent the wide variations in R_F values that sometimes occur when different samples are used.

REFERENCES

James, A. T. & Martin, A. J. P. (1952). *Biochem. J.* (in the Press).

James, A. T., Martin, A. J. P. & Howard Smith, G. (1952). *Biochem. J.* (in the Press).

The Incorporation of ^{65}Zn into Avian Eggs. By R. Tupper, R. W. E. Watts and A. Wormall. (*Departments of Biochemistry and Physiology, The Medical College of St Bartholomew's Hospital, London, E.C.* 1)

Information concerning the zinc content of avian eggs is fragmentary. We have, therefore, studied the elimination of ^{65}Zn in the eggs laid by hens injected with a ^{65}Zn-containing zinc-glycine complex prepared as described previously for studies on the labelling of red and white blood cells with this radioactive isotope (Tupper, Watts & Wormall, 1950).

The egg yolks contained appreciable amounts of ^{65}Zn, with maximum values about 6–10 days after the injection of the zinc-glycine complex. No significant amount was found in the egg white or the shell. The incompletely formed eggs were removed from one hen killed 12 days after the injection, and from another which died several hours after the injection. The highest concentration of ^{65}Zn was found in the smaller (least mature) oocytes, and it seems probable that no exchange of zinc normally occurs between the yolk and other tissues during the passage of the yolk through the bird's oviduct and uterus.

Preliminary tests have been made to determine the nature of the zinc compounds in the egg yolks examined, with particular reference to the protein fractions. The lipid fractions contained no ^{65}Zn, and virtually all the yolk zinc was present in the lipovitellin. Repeated solution of the lipovitellin in 10% (w/v) NaCl and precipitation by the addition of 20 vol. of water did not significantly alter the ^{65}Zn/total nitrogen ratio, and this and other evidence we have obtained suggests that the zinc in this fraction is firmly bound to the lipovitellin or some other protein closely associated with it. Little or no ^{65}Zn was detectable in the livetin, or in the 'phosvitin' (prepared as described by Mecham & Olcott, 1949). A further study is being made of the zinc compound or compounds present in the egg yolk protein fractions.

REFERENCES

Mecham, D. K. & Olcott, H. S. (1949). *J. Amer. chem. Soc.* **71**, 3670.

Tupper, R., Watts, R. W. E. & Wormall, A. (1950). *Biochem. J.* **48**, xxxvii.

Urinary Coproporphyrin I in Lead Poisoning. By J. E. Kench, R. E. Lane and H. Varley. (*Manchester Royal Infirmary and University of Manchester*)

The urinary lead and coproporphyrin excretion in seven bath enamellers, who showed signs and symptoms of lead poisoning, were studied during 130 days following removal from exposure to lead.

The ether-soluble porphyrins collected from all cases during this period were pooled and analysed. Single-dimensional paper chromatography by the procedure of Nicholas & Rimington (1949) showed the presence of coproporphyrin only, whilst chromatography of the derived methyl esters by the method of Chu, Green & Chu (1951) using oleic acid-kerosene (1:1) separated coproporphyrin I (29%), R_F 0·42, and coproporphyrin III (71% of the mixture), R_F 0·64. The positions of spots were checked with authentic specimens of coproporphyrin I and III methyl esters.

The methyl esters were separated using cold, dry ether, and crystallized from chloroform-methanol, giving coproporphyrin I ester, m.p. 244°, and coproporphyrin III ester, m.p. 136°.

Further urinary specimens (four cases) were collected on the 329th and 330th days, and each one examined by chromatography. Coproporphyrin I was present in all specimens, in concentrations 42, 67, 117 and 348 μg./l., ranging from 20 to 47% of the total urinary porphyrin excretion. These values confirm that the high coproporphyrin I level of the pooled extracts did not arise from a single anomalous case, but was a feature of lead intoxication.

The presence of urinary coproporphyrin I in lead poisoning has not hitherto been described, but is compatible with the increased red cell destruction and bone marrow hyperactivity observed in the disease (Watson, 1937; Dobriner & Rhoads, 1938).

This finding was discussed in relation to mechanisms which have been suggested for the action of lead on haemoglobin production (Rimington, 1938; Kench, Gillam & Lane, 1942).

REFERENCES

Chu, T. C., Green, A. A. & Chu, E. J. (1951). *J. biol. Chem.* **190**, 643.
Dobriner, K. & Rhoads, C. P. (1938). *J. clin. Invest.* **17**, 107.
Kench, J. E., Gillam, A. E. & Lane, R. E. (1942). *Biochem. J.* **36**, 384.
Nicholas, R. E. H. & Rimington, C. (1949). *Scand. J. Lab. Invest.* **1**, 12.
Rimington, C. (1938). *C.R. Lab. Carlsberg*, **22**, 454.
Watson, C. J. (1937). *J. Clin. Invest.* **16**, 383.

A Photoelectric Scanning Device for Electrophoresis of Proteins on Filter Paper. By A. L. Latner, F. Braithwaite and R. A. Nunn. (*Departments of Pathology and Surgery, The Medical School, Newcastle-upon-Tyne*)

The paper strip is prepared by a technique demonstrated at this meeting (Latner, 1952). It is rendered transparent by a method similar to one already described (Grassmann, Hannig & Knedel, 1951). The paper must be rendered absolutely bubble-free. Filtered parallel light from a constant light source is passed first through the paper and then through a slit 1 mm. in width on to a selenium cell, which is made to form a continuous circuit with a fixed low resistance.

Applied in opposition to the voltage across the resistance is another voltage obtained from a potentiometer (*A*) and a potential divider. The latter has a variable voltage across it determined by a second potentiometer (*B*). This voltage is obtained from a 2 V. battery. To indicate zero balance, a mirror galvanometer is connected between potentiometer (*A*) and the fixed resistance.

At the commencement of each complete scanning, when light is passing through a portion of the paper containing no protein, potentiometer (*A*) is set to maximum and potentiometer (*B*) is adjusted to give zero balance. After its adjustment, potentiometer (*B*) is no longer touched, and in the description which follows all reference to a potentiometer refers only to potentiometer (*A*).

The technique is to advance the paper by means of a wormscrew 2 mm. at a time and to take a reading with each advance. This is done by adjusting the potentiometer until the galvanometer reads zero. It is obvious that the maximum voltage across the potentiometer now corresponds to 100% transmission of light through the paper, and that the zero reading corresponds to complete absorption. Other readings correspond to light which has been partially absorbed. It is therefore a simple matter to calibrate the potentiometer in terms of extinction values and to take a direct reading. The distance traversed is then plotted against extinction values to give the usual electrophoretic curve, which can be analysed by a planimeter.

Good results have been obtained with protein solutions of known concentration whose electrophoretic patterns have been determined by the classical Tiselius method.

A method of continuous automatic recording is at present being devised.

REFERENCES

Grassmann, W., Hannig, K. & Knedel, M. (1951). *Dtsch. med. Wschr.* **76**, 333.
Latner, A. L. (1952). *Biochem. J.* **51**, xii.

Pyridoxal Phosphate as Coenzyme of Histaminase. By H. M. Sinclair. (*Laboratory of Human Nutrition, Churchill Hospital, Oxford*)

Histaminase may be a flavoprotein (Zeller, Stern & Wenk, 1940; Swedin, 1943; Kapeller-Adler, 1949*a*). However, Leloir & Green (1946) concluded 'that the prosthetic group of histamine oxidase is not identical with any hitherto described'.

Pyridoxal phosphate is a coenzyme for certain reactions involving the α-carbon of an α-amino-acid: decarboxylation, transamination, and tryptophan synthesis and cleavage (cf. Gunsalus, 1950). Transaminases are not active upon basic amines such as histamine; and whereas the transaminase activity of the rat lung is low, the histaminase activity is very high. During purification the coenzyme is not usually split from the apoenzyme in transaminases (Green, Leloir & Nocito, 1945) or histaminase (Leloir & Green, 1946).

Since it seemed possible that pyridoxal phosphate might be a coenzyme for histaminase, a trial was made in 1950 of the effect of therapy with pyridoxine in pregnancy toxaemia (Wilson, Sinclair & Sutherland, 1952), in which condition plasma histaminase is diminished (Kapeller-Adler, 1949*b*); therapy with pyridoxine is also stated to improve hyperemesis gravidarum (McGanity, McHenry, Van Wyck & Watt, 1949).

The following evidence is now presented that

pyridoxal phosphate is a coenzyme of histaminase. (1) Poisons that react with pyridoxal, such as semicarbazide, inhibit histaminase (cf. Zeller, 1942). (2) During purification of histaminase, pyridoxine activity remains with the enzyme. The final purified preparation contains 0·3 mg. (as pyridoxine hydrochloride) per 100 g.; since the enzyme can be purified about 180 times more by electrophoresis (Swedin, 1943), this could represent 0·54 μg./mg. of the highly purified enzyme if the impurity contains no bound pyridoxine; Green *et al.* (1945) found that 1 mg. of their highly purified glutamic-aspartic transaminase contained 0·27 μg. pyridoxal phosphate. (3) In deficiency of pyridoxine in rats, histaminase activity is diminished in skin, intestine and lung. Some restoration is achieved by addition *in vitro* of pyridoxal-5-phosphate. (4) The skin of rats deficient in pyridoxine reacts more strongly to intradermal injection of histamine than does that of controls. (5) Oral administration of an antihistamine (pyribenzamine) greatly improves the lesions of the skin and mucocutaneous junctions and the priapism in rats deficient in pyridoxine.

It is concluded that pyridoxal phosphate is a coenzyme of histaminase. The most probable explanation of the lesions of the skin and mucocutaneous junctions and the priapism in pyridoxine deficiency is that they are secondary to a vascular change produced by abnormal concentrations of histamine.

I am grateful to Miss Buxton, Miss Oehlmann and Miss Mayer for skilled assistance, and to Dr F. Bergel for pyridoxal phosphate.

REFERENCES

Green, D. E., Leloir, L. F. & Nocito, V. (1945). *J. biol. Chem.* **161**, 559.
Gunsalus, I. C. (1950). *Fed. Proc.* **9**, 556.
Kapeller-Adler, R. (1949 *a*). *Biochem. J.* **44**, 70.
Kapeller-Adler, R. (1949 *b*). *Lancet*, ii, 745.
Leloir, L. F. & Green, D. E. (1946). *Fed. Proc.* **5**, 144.
McGanity, W. J., McHenry, E. W., Van Wyck, H. B. & Watt, G. L. (1949). *J. biol. Chem.* **178**, 511.
Swedin, B. (1943). *Acta med. Scand.* **114**, 210.
Wilson, D. C., Sinclair, H. M. & Sutherland, I. (1952). To be published.
Zeller, E. A. (1942). *Adv. Enzymol.* **2**, 93.
Zeller, E. A., Stern, R. & Wenk, N. (1940). *Helv. chim. Acta*, **23**, 3.

Alanine Synthesis in Bacteria. By A. S. Fairhurst, H. K. King and Cynthia E. Sewell. (*Department of Biochemistry, University of Liverpool*)

Washed suspensions of *Bacillus subtilis* synthesize alanine from pyruvic acid and ammonia. This reductive amination takes place at the same rate under aerobic or anaerobic conditions. The rate of synthesis is half maximal at a pyruvate concentration of 0·4 M, but is almost independent of ammonia concentration over the range 0·03–0·5 M. Activity falls off sharply at either side of pH 7. The pH at which the cells are grown is important, activity being maximal if the medium is neutral or slightly acid, but declining sharply at alkaline pH. Alanine synthesis proceeds, though much less readily, if ammonia is replaced by certain amino-acids, viz. arginine, glutamic, histidine, leucine, methionine, phenylalanine and valine; little or no synthesis takes place from aspartic acid, cysteine, glycine, lysine, serine, threonine or tryptophan.

Though the optimal concentration of pyruvic acid appears to be far above the physiological level, the reaction seems to take place under conditions of normal growth. Sufficient alanine is found for direct observation on paper chromatograms in the culture filtrates from media containing ammonium lactate as main carbon source with *Bacillus subtilis*, *B. polymyxa*, *B. firmus*, *Mycobacterium phlei*, *Streptococcus lactis* and *Pasteurella pseudotuberculosis*, whilst *Escherichia coli*, *Bacillus pumilus*, *Aerobacter aerogenes* and *Pseudomonas pyocyaneus* give negative results. With glucose as main carbon source, *Bacillus subtilis* and *Streptococcus lactis* shed considerable amounts of alanine into the medium: the other organisms give no more than traces.

Homogentisic Acid an Intermediate in the Metabolism of Tyrosine by the Aromatic Ring-Splitting Micro-organisms. By J. D. Jones, B. S. W. Smith and W. C. Evans. (*Department of Agricultural Chemistry, School of Agriculture, University College of North Wales, Bangor*)

When the aromatic ring-splitting bacteria (*Vibrio* 0/1, *Pseudomonas fluorescens* strains A 3/8 and A 3/9) are grown in a tyrosine mineral-salt medium under aeration, the presence of the following substances can be demonstrated chromatographically in the culture fluid: homogentisic acid,* *p*-hydroxyphenylpyruvic acid,* *p*-hydroxyphenylacetic acid and *p*-hydroxybenzoic acid. In young cultures

homogentisic acid predominates. Cells of these organisms grown on tyrosine media are completely adapted to homogentisic acid, indicating that this compound appears in the metabolic pathway of the amino-acid; the subsequent fate of this intermediate is being investigated.

As far as we are aware, this is the first instance of such a pathway occurring in bacterial metabolism, and it is possibly analogous to that recently demonstrated in mammalian liver by Knox & LeMay-Knox (1951).

* We wish to thank Dr A. Neuberger, F.R.S., for authentic samples of these compounds.

REFERENCE

Knox, W. E. & LeMay-Knox, M. (1951). *Biochem. J.* **49**, 686.

DEMONSTRATIONS

A Flame Photometer for Routine Biochemical Use. By R. L. Warren (introduced by C. F. M. Rose). (*Courtauld Institute of Biochemistry, The Middlesex Hospital Medical School, London, W.* 1)

A Routine Estimation of Sodium and Potassium in Biological Fluids by the Method of Flame Photometry. By Anne E. Moore (introduced by C. F. M. Rose). (*Courtauld Institute of Biochemistry, The Middlesex Hospital Medical School, London, W.* 1)

An Apparatus for Electrophoresis on Filter Paper. By A. L. Latner. (*Section of Chemical Pathology, Department of Pathology, Royal Victoria Infirmary, Newcastle-upon-Tyne*)

The apparatus is constructed entirely of Perspex. It consists essentially of a rectangular base 34 × 42 cm. and 12·5 mm. thick. Two rectangular compartments, each 5 × 27 × 4 cm., are recessed into it. They are parallel with its shorter side and are placed symmetrically. A distance of 25 cm. separates them, and each is further subdivided by a vertical partition along its long axis into two compartments. The outer distal ones serve for the carbon electrodes and the inner for the ends of the filter-paper strips.

The electrode and paper sections are each filled with buffer and connected with each other by cotton-wool wicks passing through four small holes drilled at equal distances along the top of the partitions between the two sections (Flynn & De Mayo, 1951).

A Perspex partition 9 cm. high and 27 cm. long is erected 4·5 cm. from the cathode compartment and parallel with it. A waxed glass rod is held in position just over the inner edge of the anode compartment. The paper strip bridges from the cathode over the Perspex partition and then over the waxed glass rod into the anode compartment, forming a gentle slope down which the electrophoresis is carried out. The paper is held in position by glass T-pieces filled with lead shot.

The whole is covered with a rectangular lid 40 × 32 cm. and 10 cm. deep, which fits into a rectangular groove carved out in the Perspex base. This is filled with mercury, which acts as an effective seal.

As many as ten electrophoretic analyses may be carried out simultaneously. Current is supplied by a 120 V. dry battery. A voltmeter and a milliammeter are incorporated in the apparatus.

The actual technique of electrophoresis was demonstrated. An essential point is the fact that the serum or protein solution is applied with a paint brush in the form of a narrow band across the dry strip of paper 2 cm. wide. The strip is then treated with buffer.

REFERENCE

Flynn, F. V. & De Mayo, P. (1951). *Lancet*, ii, 235.

A Micro-electrophoresis Apparatus. By D. N. Baron. (*Courtauld Institute of Biochemistry, The Middlesex Hospital Medical School, London, W.* 1)

[*The Editors of the Biochemical Journal accept no responsibility for the Reports of the Proceedings of the Society. Abstracts of papers read are published as received from the authors.*]

PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **305th Meeting** *of the Biochemical Society was held in the London School of Hygiene and Tropical Medicine, Keppel Street, London, W.C.* 1, *on Saturday,* 16 *February* 1952, *and took the form of a Symposium on 'Lipid Metabolism'.*

COMMUNICATIONS

The Mechanism of Fat Absorption. By A. C. FRAZER. (*Department of Pharmacology, University of Birmingham*)

Recent work lends strong support to the partition hypothesis (Frazer, 1952).

Intraluminar phase. Hydrolysis. Long-chain triglycerides are only partially hydrolysed to fatty acids and lower glycerides *in vivo* as well as *in vitro*. Short-chain compounds are more rapidly and more completely hydrolysed. Fat is absorbed in two forms—water-insoluble glyceride or water-soluble fatty acid or soap.

Emulsification. Long-chain glycerides are finely emulsified in the intestinal lumen by the system—bile salts/fatty acids/lower glycerides. Emulsification does not occur in the absence of lipase or bile salts. Tributyrin and fatty acid need not be finely emulsified for absorption to occur. Paraffin alone is not hydrolysed, emulsified or absorbed; if paraffin is finely emulsified in the intestinal lumen, extensive absorption occurs.

Cellular phase. Fat fills the fine canaliculi in the 'brush border' of the intestinal cells during absorption. Resynthesis of triglycerides can occur in the intestine.

Phosphorylation. Phospholipin is formed in the intestine and facilitates fat absorption. It is not an obligatory intermediate in the fat absorption mechanism (Zilversmit, Chaikoff & Entenman, 1948). Iodoacetic acid intoxication interferes with gastro-intestinal motility, but not with fat absorption. Adrenalectomy interferes with the absorption of long-chain, but not of short-chain, fats.

Distributive phase. Long-chain saturated fats. When liquid fat is fed, 85 % or more passes in the chyle; with insufficient carrier glyceride, absorption is poor.

Short-chain fats. There is low percentage recovery in chyle; they may pass by the portal route.

Unsaturated fats. Glycerides normally pass in the chyle; fatty acids variable.

REFERENCES

Frazer, A. C. (1952). *Avery-Jones' Modern Trends in Gastroenterology.* London: Butterworth.

Zilversmit, D. B., Chaikoff, I. L. & Entenman, C. (1948). *J. biol. Chem.* **172**, 637.

Comparative Aspects of Lipid Absorption and Excretion. By R. P. COOK. (*Department of Biochemistry, University College, Dundee*)

Values for the absorption of various natural fats at different levels in the diets of several animal species were presented. In mammals absorption is generally of a high order (90–98 %), the guinea pig showing poor absorption with high levels of fat. The physical and chemical properties of the fatty acids affect their absorption.

Sterols form a small percentage of the total ingested lipid, but dietary cholesterol has been implicated as a causative agent in the pathogenesis of atherosclerosis and possibly of coronary artery disease. The evidence for this is based largely on experiments with the rabbit which has a high level of absorption for cholesterol. In man, absorption of crystalline cholesterol is low, but when given in eggs approximately half of the cholesterol is absorbed. A transient increase in the plasma level can be demonstrated. Most of the other sterols are poorly absorbed.

Lipids are excreted in the urine, faeces and sebum. The lipid components of these excretions were discussed. It is suggested that one of the roles of the lipids in the intestine, as well as in skin, is to act as a conditioning agent.

Defective Fat Absorption in Man. By J. M. FRENCH. (*Department of Pharmacology, University of Birmingham*)

Fat absorption is best assessed by estimating daily fatty acid output on measured intake (Kamer, Huinink & Weyers, 1949). Whether faecal fat is derived from dietary fat, bacterial synthesis or excretion is determined by studying the effect of varying fat intake and antibiotics.

Intraluminar changes are examined by intubation and fat is normally found to be finely emulsified. Absorption of finely dispersed glycerides gives rise to a characteristic hyperlipaemia. The absorptive capacity of the upper small intestine is assessed by intraduodenal glucose absorption curves. The normal small intestine gives a feathery radiographic pattern, which can be changed to the flocculation pattern in normal subjects by administration of fatty acids, which increase mucus secretion (Frazer, French & Thompson, 1949).

For pancreatic syndrome (PS) and sprue syndrome (SS) the following observations have been made:

Fat absorption: PS, 48% (range 15–68%, 5 cases); SS, 73% (range 54–86%, 24 cases).

Pancreatic enzymes: PS, deficient, <1 unit; SS, normal, 10 units.

Emulsification: PS, faulty, particles $>10\,\mu$.; SS, normal, particles $<0{\cdot}5\,\mu$.

Chylomicrographs. (*a*) Unemulsified oil: PS, flat, max. <10 particles per standard field; SS, flattened, max. <50 particles per standard field. (*b*) Emulsified oil: PS, normal, max. >120 particles per standard field.

Glucose and urea: PS, normal absorption; SS, depressed and delayed.

Radiography: PS, normal feathery pattern; SS, flocculation pattern.

Pancreatic enzyme deficiency. Lipolysis is essential for intraluminar emulsification. Normal glyceride absorption is dependent on fine emulsification. Fatty acid effects are absent until pancreatin is administered, when a radiographic flocculation pattern appears (Lowe, May, Stauffer & Neuhauser, 1950).

Sprue syndrome. Intraluminar changes are normal, but intestinal absorption is depressed. Delayed glyceride absorption leads to increased fatty acid, which causes increased mucus secretion and the radiographic flocculation pattern. Increased mucus secretion, decreased motility and altered intestinal flora may all play a part in the aetiology of the fat absorption defect in the sprue syndrome (Frazer, 1951).

REFERENCES

Frazer, A. C. (1951). *Parsons' Modern Trends in Paediatrics.* London: Butterworth.

Frazer, A. C., French, J. M. & Thompson, M. D. (1949). *Brit. J. Radiol.* **22**, 123.

Kamer, J. H. van de, Huinink, ten B. & Weyers, H. A. (1949). *J. biol. Chem.* **177**, 347.

Lowe, C. U., May, C. D., Stauffer, H. M. & Neuhauser, E. D. B. (1950). *Amer. J. Dis. Child.* **79**, 91.

Lipid Synthesis from Small Molecules. By G. POPJÁK. (*National Institute for Medical Research, Mill Hill, London, N.W. 7*)

Ever since Lawes & Gilbert demonstrated in 1866 the conversion of carbohydrate to fat, there has been an intensive search to establish the nature of the intermediates of this conversion. The idea that a two-carbon compound might be the building stone for fatty acid synthesis originated perhaps at the time when Nencki demonstrated in 1878 that two molecules of acetaldehyde in an alkaline solution will condense to form aldol, and Magnus-Levy (1902) suggested that the higher fatty acids might be built up by the repeated condensation of acetaldehyde (cf. Raper, 1907). The earliest demonstration of both fat and sterol synthesis from acetate was made by Smedley MacLean & Hoffert (1926) who found the accumulation of these substances in yeast cells grown in a medium which contained only sodium acetate as a carbon source. The importance of C_2 compounds in the synthesis of animal fats, however, was not firmly established until after 1935, when Schoenheimer and his colleagues introduced isotopic tracers for the study of this problem (cf. Schoenheimer, 1941). Bloch & Rittenberg (1942*a*, *b*) showed that the small molecules from which both fatty acids and cholesterol are synthesized in animals are acetate units or a more reactive derivative of acetate. The central role of acetate in the intermediary metabolism of fat has since been firmly established.

In some recent investigations, in which the lactating mammary gland has been used for the study of fatty acid synthesis (Popják, French & Folley, 1951; Popják, French, Hunter & Martin, 1951; see also Dr Folley's contribution to this Symposium), the mode of condensation of acetate

units to form the fatty acid chains has been established. It was found that the fatty acid chains are formed by the stepwise lengthening of a shorter acid at the carboxyl end by the addition of an acetate unit.

In studies on the conversion of carbohydrate to fat (French & Popják, 1951), it was shown that glucose was as good a source of carbon for the formation of both fatty acids and cholesterol as acetate. Further, it was found that glucose is broken down in rabbits very efficiently to acetate, at least 25% of the body acetate being derived from glucose. It appears probable from experimental evidence (Popják & Hunter, unpublished) that the conversion of glucose to fat involves the breakdown of glucose to pyruvate, which on oxidative decarboxylation yields acetate.

The importance of carbohydrate as a source of fat is that glucose not only gives rise to intermediates necessary for fatty acid synthesis, but is also a direct source of glycerol.

REFERENCES

Bloch, K. & Rittenberg, D. (1942*a*). *J. biol. Chem.* **143**, 297.

Bloch, K. & Rittenberg, D. (1942*b*). *J. biol. Chem.* **145**, 625.

French, T. H. & Popják, G. (1951). *Biochem. J.* **49**, iii.

Lawes, J. B. & Gilbert, J. H. (1866). *Phil. Mag.* IV, **32**, 439.

Magnus-Levy, A. (1902). *Arch. Anat. Physiol.*, Physiol. Abt., 365.

Nencki, M. (1878). *J. prakt. Chem.* II, **17**, 105.

Popják, G., French, T. H. & Folley, S. J. (1951). *Biochem. J.* **48**, 411.

Popják, G., French, T. H., Hunter, G. D. & Martin, A. J. P. (1951). *Biochem. J.* **48**, 612.

Raper, H. S. (1907). *J. chem. Soc.* **91**, 1831.

Schoenheimer, R. (1941). *The Dynamic State of Body Constituents.* Cambridge, Mass.: Harvard University Press.

Smedley MacLean, I. & Hoffert, D. (1926). *Biochem. J.* **20**, 343.

Aspects of Fat Metabolism in the Ruminant with Special Reference to the Biosynthesis of Milk Fat. By S. J. FOLLEY. (*National Institute for Research in Dairying, University of Reading*)

Formerly only fat and carbohydrate (hexose) were considered as sources of depot and milk fat arising from dietary digestion in ruminants. Recent work has shown that rumen micro-organisms break down food polysaccharides to lower fatty acids, mainly acetic acid, which enter the blood in significant amounts. Acetate of exogenous origin, mixing with endogenous C_2 fragments, is thus available as a substrate for milk fatty acid synthesis.

An important question is: does the synthesis of milk fatty acids occur in the mammary gland itself? Arterio-venous studies show that blood glycerides are absorbed by the lactating udder. If pre-formed glycerides provide the sole source of milk fat, then the short-chain fatty acids (C_4–C_{12}) characteristic of the milk fat of many herbivorous species must arise by degradation of long-chain acids in the udder, as postulated by Hilditch. However, the high R.Q. of the lactating udder *in vivo* and of surviving udder slices suggests that mammary tissue can synthesize fat from small, oxygen-rich molecules, such as carbohydrate or acetate.

Experiments with $CH_3\,{}^{14}COOH$ have shown conclusively that acetate is a major source of carbon for fatty acid formation in the udder, and that the milk short-chain acids arise not by degradation of oleoglycerides, but are intermediates in the formation of long-chain acids by stepwise chain elongation. Milk fatty acids thus have a dual origin; some are synthesized in the udder from small molecules, others enter the gland from the blood and may thereafter be partly degraded.

Glycerol, a limiting factor in glyceride and hence fatty acid formation, can arise in the udder from breakdown of hexose; since the udder incorporates acetate carbon into lactose (and thus hexose), blood acetate is an indirect precursor of glycerol—an additional and more direct precursor must be the blood glucose.

The Enzymic Oxidation of Fatty Acids. By A. L. LEHNINGER. (*Department of Biochemistry, University of Chicago*)

The fatty acid oxidase system, as found in isolated rat-liver mitochondria, requires for activity Mg^{++}, adenosinetriphosphate, an optimum osmolar concentration of electrolytes or certain non-electrolytes such as sucrose, and an oxidizable 'priming' or 'sparking' metabolite, which may be a Krebs citric acid cycle intermediate. The function of the latter appears to lie in its ability to cause oxidative

phosphorylation. The presence of a cycle intermediate *per se* is however not necessary, since reduced cozymase (which undergoes phosphorylative oxidation) activates the oxidation equally well.

When cycle intermediates are not present, oxidation of fatty acids proceeds quantitatively to acetoacetate. In their presence, variable proportions of the fatty acid are oxidized to completion via the cycle. The longer the chain the greater is the proportion of fatty acid carbon recovered as CO_2.

Early isotope experiments indicated that acetoacetate was formed by essentially random condensation of acetyl residues. More recent refinements of this approach indicate that more than one molecular species of active acetyl may be formed from the fatty acid chain, depending on the locus of origin, and that these may differ in ability to serve as precursors of the 'head' and 'tail' of acetoacetate. Other mechanisms may be invoked to explain the formation of 'asymmetric' acetoacetate from labelled fatty acid.

The most revealing approach to the mechanism of the primary oxidative attack is furnished by recent work on extracts of *Clostridium kluyveri*.

Essential Fatty Acids and their Relation to Pyridoxine. By H. M. SINCLAIR. (*Laboratory of Human Nutrition, University of Oxford*)

Six different fatty acids have been shown to be effective in curing lesions in rats, and deficiency of fatty acids has been shown to occur also in the chick, dog, mouse and probably the bovine, pig and man.

Burr & Barnes (1943) have characterized the changes as: '(1) development of scaly skin and caudal necrosis; (2) retardation of growth; (3) kidney lesions and hematuria; and (4) early death.' Sterility, high R.Q., high metabolic rate and high water consumption also occur. This is an erroneous summary of the pathological changes, which will be discussed.

The pathological lesion in the skin of rats in deficiency of essential fatty acids is grossly similar to that found in deficiency of pyridoxine; that there might be a relation between the two deficiencies was first indicated by Hogan & Richardson (1934). Histologically the two lesions are very different. Whereas deficiency of either nutrient alone produces in young rats a failure of growth and acrodynia, these two signs are more apparent if both nutrients are deficient. Yet neither nutrient will cure a pure deficiency of the other. Further, in fatty-acid deficiency the concentration of pyridoxine in the body is found to be normal.

As a working hypothesis it is suggested that there is failure of growth in fatty-acid deficiency because phosphatides and therefore cell membranes cannot be formed, whereas in pyridoxine deficiency the cause is interference with protein metabolism through deficient transamination and decarboxylation; also it is suggested that the skin lesions in fatty-acid deficiency allow water to pass through the skin more readily than normally, whereas in pyridoxine deficiency there is a deficiency of histaminase and therefore inadequate destruction of histamine.

REFERENCES

Burr, G. O. & Barnes, R. H. (1943). *Physiol. Rev.* **23**, 256.

Hogan, A. G. & Richardson, L. R. (1934). *J. Nutrit.* **8**, 385.

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PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **Annual General Meeting** *of the Biochemical Society was held at the National Institute for Medical Research, Mill Hill, London, N.W. 7, on Friday, 21 March 1952, when the following papers were read:*

COMMUNICATIONS

Formation of Citrulline by *Staphylococcus aureus* in an Arginine-containing Medium. By I. Lominski, R. B. Morrison and I. A. Porter (introduced by J. N. Davidson). (*Department of Bacteriology, The University and Western Infirmary, Glasgow*)

Tsutomu Sekine (1947), and more recently Knivett (1952), reported the formation of citrulline from arginine by *Streptococcus faecalis*.

In the course of work on the breakdown of amino-acids by Staphylococci a similar finding was noted. The strain used was a coagulase-positive *Staphylococcus aureus* of human origin. The work was carried out in a chemically defined medium containing alanine, aspartic acid, arginine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, valine, aneurin, biotin, nicotinamide, pyridoxin, magnesium sulphate and potassium phosphate; the constituents were dissolved in citric acid/phosphate buffer, pH 7·3.

An 18-hour-old broth culture of *Staph. aureus* was centrifuged, washed and subcultured three times in the chemically defined medium before serving as inoculum for the experiments. The blank medium and cultures were incubated and examined at intervals by paper chromatography. The density of the cultures was approximately $3{\cdot}5 \times 10^8$ cells/ml. on the 3rd day and 7×10^8 cells/ml. on the 7th day of incubation. Between the 5th and 7th day of incubation a citrulline spot appeared in the chromatogram; this spot was more intense in the 9 day chromatogram. The arginine spot simultaneously showed fading in intensity. No citrulline was detected in the blank medium, in young cultures, and when norleucine or ornithine was substituted for arginine in equimolar proportion.

It is possible that the breakdown of arginine by *Staph. aureus* is similar to that of *Strep. faecalis* since citrulline is not formed in the absence of arginine and the appearance of citrulline is accompanied by the disappearance of arginine.

REFERENCES

Knivett, V. A. (1952). *Biochem. J.* **50**, xxx.
Tsutomu Sekine (1947). *J. Biochem.* **19**, 79 (quoted from *Chem. Abstr.* (1950), **44**, 10789*g*.

The Occurrence of Fructose in Rabbit-Liver Glycogen. By S. Peat, P. J. P. Roberts and W. J. Whelan. (*Chemistry Department, University College of North Wales, Bangor*)

In the course of a study of α-amylolysis, we have had occasion to use glycogen as one of the substrates for the action of salivary α-amylase. The glycogen was isolated from the livers of pregnant does and the α-amylase was a purified freeze-dried specimen (cf. Roberts & Whelan, 1951). The end products of its action on this glycogen (6·155 g.) were submitted to fractionation on a charcoal column (cf. Bailey, Whelan & Peat, 1950). There was no monosaccharide fraction, and the first disaccharide component to be eluted from the column was not maltose. It was a copper-reducing disaccharide (yield, 0·289 g.) showing $[\alpha]_D + 54{\cdot}6°$ in water (maltose has $[\alpha]_D + 136°$) and resisted oxidation by alkaline hypoiodite. Under the conditions employed, this reagent effected some oxidation of fructose (0·102 mole I_2 consumed) and the new disaccharide was oxidized to the same extent (0·104 mole I_2). Glucose and maltose consumed 1·02 and 0·993 mole I_2, respectively. Nevertheless, the new disaccharide formed a phenylosazone (method of Hassid & McCready, 1942) identical in crystalline habit and specific rotation ($[\alpha]_D + 80{\cdot}6°$ in pyridine-ethanol) with maltosazone ($[\alpha]_D + 79{\cdot}2°$). It was hydrolysed (96%) by yeast α-glucosidase, but not by invertase (β-fructofuranosidase). Paper chromatography of the hydrolysate indicated the presence of fructose as well as glucose. From this hydrolysate 2:3–4:5-diacetone fructose, m.p. 90°, $[\alpha]_D - 32{\cdot}8°$ in water was isolated, as by Bacon & Bell (1948); an authentic specimen had m.p. 91°,

$[\alpha]_D - 32{\cdot}9°$. The sugar thus appeared to be 4-[α-glucopyranosido]-fructose (maltulose). This structure has been confirmed by the synthesis of maltulose from maltose. The latter (11·64 g.) on treatment with lime water (35°; 24 hr.) gave a product (1·03 g., isolated by charcoal chromatography) having $[\alpha]_D + 52{\cdot}8°$ in water (phenylosazone, $[\alpha]_D + 75°$). It consumed only 0·078 mole I_2. When it was hydrolysed by acid, it showed a reducing power/time relationship identical with that given by the new disaccharide; after an initial increase, reducing power decreased in linear fashion due to the destruction of fructose by the acid.

A comparison was also made with 3-[α-glucopyranosido]-fructose, i.e. turanose, hitherto the only known reducing glucosido-fructose. Turanose ($[\alpha]_D + 75{\cdot}8°$ in water) is oxidized to a greater extent by hypoiodite (0·252 mole I_2 consumed) and its phenylosazone has $[\alpha] + 33{\cdot}1°$ in pyridine-ethanol.

REFERENCES

Bacon, J. S. D. & Bell, D. J. (1948). *Biochem. J.* **42**, 397.
Bailey, J. M., Whelan, W. J. & Peat, S. (1950). *J. Chem. Soc.* p. 3692.
Hassid, W. Z. & McCready, R. M. (1942). *Industr. Engng Chem.* (Anal. ed.), **14**, 683.
Roberts, P. J. P. & Whelan, W. J. (1951). *Biochem. J.* **49**, lvi.

Action Pattern of Salivary α-Amylase. By P. J. P. Roberts and W. J. Whelan. (*Chemistry Department, University College of North Wales, Bangor*)

Roberts & Whelan (1951) have shown that the end products of the action of pure salivary α-amylase on amylose are maltose and maltotriose in the molecular proportion 2·39:1. The hypothesis was advanced by Meyer & Gonon (1951) that α-amylase acts on amylose by the random scission of all except the terminal α-1:4-linkages. This action gives rise to the above sugars, which contain only 'terminal' linkages. These authors report, however, that maltotriose is slowly hydrolysed by α-amylase to maltose and glucose, a fact which appears to be at variance with their hypothesis of resistant terminal linkages. Using the statistical treatment of this action as developed by Meyer & Gonon, we showed that the calculated ratio of maltose:maltotriose should be 2·35:1, in good agreement with the experimentally observed value.

Further evidence has now been obtained in support of the hypothesis. Four maltosaccharides, namely, malto-tetraose, -pentaose, -hexaose and -heptaose, prepared as described by Bailey, Whelan & Peat (1950), have been subjected to α-amylolysis (salivary enzyme) and the end products fractionated on a charcoal column. Complete separation was obtained, 20–30 mg. of each maltosaccharide were used and no products other than maltose and maltotriose were detected. Yields were corrected for the respective recoveries of 94·4 and 95·2 % observed in a control fractionation of an artificial mixture of maltose and maltotriose on charcoal. Maltotetraose: after α-amylolysis, R_{Cu} (reducing power as % maltose) was 99·4 (calc. 100·0) and D/T (maltose: maltotriose molecular ratio, from weight yields) was 2·00:0 (calc. 2·00:0). Maltopentaose: after α-amylolysis, R_{Cu} was 79·4 (calc. 80) and D/T was 1·00:1 (calc., 1·00:1). Maltohexaose: after α-amylolysis, R_{Cu} was 86·1 (calc. 88·8) and D/T was 2·98:1 (calc. 3·00:1). Maltoheptaose: after α-amylolysis, R_{Cu} was 84·6 (calc., 85·7) and D/T was 2·00:1 (calc. 2·00:1).

We are unable to confirm the slow α-amylolysis of maltotriose which Meyer & Gonon report, and are convinced that our specimens of salivary α-amylase are entirely without action on this substrate.

REFERENCES

Bailey, J. M., Whelan, W. J. & Peat, S. (1950). *J. chem. Soc.* p. 3692.
Meyer, K. H. & Gonon, W. F. (1951). *Helv. chim. Acta*, **34**, 294, 308.
Roberts, P. J. P. & Whelan, W. J. (1951). *Biochem. J.* **49**, lvi.

Ionophoresis of Sugars on Paper and Some Applications to the Analysis of Protein-Polysaccharide Complexes. By R. CONSDEN and WINIFRED M. STANIER. (*Canadian Red Cross Memorial Hospital, Taplow, Maidenhead, Berks.*)

Sugars can form complexes with borate ions (Böeseken, 1949), and differences in properties of borate complexes of certain sugars or their derivatives have enabled separations to take place on chromatograms (Khym & Zill, 1951; Annison, James & Morgan, 1951). In solutions containing borate, we have found that sugars migrate towards the anode, when a voltage is applied. Ionophoresis of a series of sugars was carried out on Whatman no. 1 paper at a potential of 10 V./cm. in buffers containing borate. Mobilities were negligible at pH6 and increased with increase in pH. For most of the sugars, a maximum was reached above pH 9·2. Differences between the mobilities of sugars at optimum pH enables various separations to be achieved. After ionophoresis, further resolution of a mixture may be brought about by chromatography at right angles to the direction of ionophoresis, thus producing a two-dimensional separation (cf. Haugaard & Kroner, 1948). This method is especially useful in examining hydrolysates of proteins containing only small amounts of polysaccharide, since the sugars, because of their relatively high mobility at certain pH values, can be initially separated, by ionophoresis, from much of the peptide and amino-acid material, which would interfere with their chromatography. The same sheet may be examined for amino-acids and peptides by treatment with ninhydrin, after the sugar spots have been revealed with aniline hydrogen phthalate (Partridge, 1949). Protein-polysaccharide hydrolysates were initially subjected to ionophoresis in borate at pH 8·6. At this pH, the hexosamines behave as neutral substances, whilst glucuronic acid has a higher mobility than any of the sugars.

Examination of a hydrolysate of group A haemolytic streptococcus revealed at least four sugars, which behaved ionophoretically and chromatographically like hexosamine, rhamnose, ribose and glucose (cf. Schmidt, 1952). Rhamnose was present in highest amount, whilst ribose, which was relatively weak, varied in amount with different strains.

Human fibrin, purified by the method of Lorand (1951), which on hydrolysis liberated 2·7 % reducing sugar and 0·6 % hexosamine, gave hexosamine, mannose and galactose. Thus, fibrin contains several per cent of bound polysaccharide consisting of these three components. Werner & Odin (1949), by paper chromatographic examination, have recorded similar results for other blood proteins.

REFERENCES

Annison, E. F., James, A. T. & Morgan, W. T. J. (1951). *Biochem. J.* **48**, 477.
Böeseken, J. (1949). *Advances in Carbohydrate Chemistry*, **4**, 189.
Haugaard, G. & Kroner, T. D. (1948). *J. Amer. chem. Soc.* **70**, 2135.
Khym, J. X. & Zill, L. P. (1951). *J. Amer. chem. Soc.* **73**, 2399.
Lorand, L. (1951). *Nature, Lond.*, **167**, 992.
Partridge, S. M. (1949). *Nature, Lond.*, **164**, 443.
Schmidt, W. C. (1952). *J. exp. Med.* **95**, 105.
Werner, I. & Odin, L. (1949). *Experientia*, **5**, 233.

Cocarboxylase and Protein-bound Aneurin in Relation to Phosphatase in Colostrum and Milk of Cows. By R. CHANDA and E. C. OWEN. (*The Hannah Dairy Research Institute, Kirkhill, Ayr*)

Houston, Kon & Thompson (1940) showed that aneurin occurs in the milk of the cow in at least three distinct forms. the free vitamin, cocarboxylase and a protein-bound form. They measured the concentration of free and total aneurin in the milk throughout lactation and stated that there was a marked negative correlation between phosphatase and cocarboxylase. Later Chanda, McNaught & Owen (1949) showed that injection of thiouracil into lactating cows had little effect on total aneurin in the milk but caused a marked decrease in both its phosphorylated and protein-bound forms. Thyroxine produced the reverse of both these effects. It was therefore thought that protein-bound aneurin might vary inversely as phosphatase during lactation in much the same way as Houston *et al.* (1940) had shown for cocarboxylase. An investigation was therefore made of the protein-bound aneurin in cow colostrum and milk. At the same time the total aneurin and cocarboxylase were estimated, the methods of analysis being the same as those of Houston *et al.* (1940). The results showed that the changes of both protein-bound aneurin and cocarboxylase were negatively correlated with phosphatase in both colostrum and milk. Thus in the first post-partum secretion cocarboxylase was 27 %

of the total aneurin but rose to 60 % by the third day. The percentage remained steady for about the next 4 days but slowly decreased thereafter, so that by the 38th week of lactation it was only 8 %. The first colostrum had 19 % of the aneurin in the protein-bound form. By the second day the percentage had fallen to 9. It exhibited a second maximum of 16 % on the fourteenth day. Thereafter there was a steady decrease of the protein-bound form throughout lactation. In the 38th week the value was 6 %. Immediately *post partum* the phosphatase level was 154 units but had fallen to 17 by the third day. From the fourth week onwards the phosphatase gradually increased to 189 units in the 38th week. Total aneurin showed a decrease throughout lactation, colostrum and transitional milk being much richer than milk secreted later. These observations showed that protein-bound aneurin, resembles ester P and lipid P in cows' milk (Chanda & Owen, 1951), cocarboxylase in goat and cow milk (Houston *et al.* 1940), bound aneurin in sow milk (Braude, Coates, Henry, Kon, Rowland, Thompson & Walker, 1947) and ester P, lipid P and cocarboxylase in human milk (Chanda, Owen & Cramond, 1951), in being negatively correlated with phosphatase.

REFERENCES

Braude, R., Coates, M. E., Henry, K. M., Kon, S. K., Rowland, S. J., Thompson, S. Y. & Walker, D. M. (1947). *Brit. J. Nutrit.* **1**, 64.

Chanda, R., McNaught, M. L. & Owen, E. C. (1949). *Biochem. J.* **45**, xix.

Chanda, R. & Owen, E. C. (1951). *Biochem. J.* **50**, 100.

Chanda, R., Owen, E. C. & Cramond, B. (1951). *Brit. J. Nutrit.* **5**, 228.

Houston, J., Kon, S. K. & Thompson, S. Y. (1940). *J. Dairy Res.* **11**, 145.

The Effect of Cortisone and Adrenocorticotropic Hormone on the Dehydroascorbic Acid of Human Blood Plasma. By C. P. Stewart, D. Horn and J. S. Robson. (*Department of Clinical Chemistry, University of Edinburgh*)

Ascorbic acid was determined photometrically in blood plasma by means of 2:6-dichlorophenol-indophenol. Ascorbic acid plus dehydroascorbic acid was determined by the method of Roe & Keuther (1943). The results suggested that plasma contained dehydroascorbic acid in variable amount averaging about 20 % of the ascorbic acid. When plasma was treated with hydrogen sulphide, the two methods gave identical results, and the whole of the apparent ascorbic acid was rapidly removed by cucumber oxidase.

From this and from various control experiments—recovery of added ascorbic acid, effect of time on the plasma ascorbic acid content, effect of such alleged stabilizers as thiourea and cyanide—it is concluded that the two methods, used simultaneously, do indicate the presence of dehydroascorbic acid in normal human plasma.

The plasma concentration of dehydroascorbic acid fell to zero when cortisone was given orally, so that 1–2 hr. afterwards the two methods gave identical results. Thereafter dehydroascorbic acid reappeared. There was no increase in the total plasma ascorbic acid + dehydroascorbic acid.

Adrenocorticotropic hormone given parenterally in comparable dosage, produced, rapidly and temporarily, a similar diminution in the dehydroascorbic acid of the plasma, but also an actual increase in the total.

REFERENCE

Roe, J. H. & Keuther, C. A. (1943). *J. biol. Chem.* **147**, 399.

The Assay of β-Glucuronidase in Rat Liver. By P. G. Walker and G. A. Levvy. (*Rowett Research Institute, Bucksburn, Aberdeenshire*)

Whereas the total glucuronidase activity, soluble and insoluble, in water homogenates of mouse liver could be measured directly, provided that the preparation was not unduly concentrated (Kerr & Levvy, 1951; Walker & Levvy, 1951), this was not true of rat liver which has been found to contain a thermostable, non-dialysable inhibitory factor, apparently similar to that observed by Fishman, Altman & Springer (1948) in blood plasma. Inhibition was overcome by adding the surface-active

agent, Triton X-100 (Rohm and Haas Co.), permitting measurements of the full glucuronidase activity in all types of preparation.

On fractionation of unbuffered water homogenates of rat liver on the high-speed centrifuge, both the soluble and insoluble enzyme fractions contained inhibitor. On fractionation by buffering to pH 5·2, all the inhibitor sedimented with the insoluble enzyme, the activity of which was greatly depressed. Enzyme-free inhibitor obtained by boiling caused up to 75% inhibition (non-competitive) of inhibitor-free soluble enzyme.

The effect of endogenous inhibitor in the homogenate was more pronounced in infant than in adult rat liver, and ruled out the possibility of direct assay in homogenates in absence of Triton X-100. After extraction of the soluble enzyme in acetate buffer, it displayed a higher activity than the homogenate, being inhibitor-free, but represented a variable fraction of the true total activity of the tissue, depending among other things on the age of the animal.

Measurements of true total glucuronidase activity in rat-liver homogenates made in the presence of Triton X-100 showed a rise during the first month after birth, and a subsequent fall in the case of males, but not females. There would thus appear to be a species difference between the rat and the mouse in the variation in liver glucuronidase activity with age (see Levvy, Kerr & Campbell, 1948; Mills, Smith, Stary & Leslie, 1950).

The effect of endogenous inhibitor was also pronounced in rat-kidney homogenates, but was weak in rat spleen. Rat-spleen glucuronidase was, however, powerfully inhibited by the factor in liver. Mouse liver and kidney contained powerful inhibitors for the enzyme in rat tissues, but mouse glucuronidase was not susceptible to the action of inhibitors from any mouse or rat tissue. Increasing the inhibitor concentration increased the percentage inhibition of a given enzyme preparation only up to a certain maximum which was characteristic of the source of both the enzyme and the inhibitor. This finding is still unexplained.

REFERENCES

Fishman, W. H., Altman, K. I. & Springer, B. (1948). *Fed. Proc.* **7**, 154.

Kerr, L. M. H. & Levvy, G. A. (1951). *Biochem. J.* **48**, 209.

Levvy, G. A., Kerr, L. M. H. & Campbell, J. G. (1948). *Biochem. J.* **42**, 462.

Mills, G. T., Smith, E. E. B., Stary, B. & Leslie, I. (1950). *Biochem. J.* **47**, xlviii.

Walker, P. G. & Levvy, G. A. (1951). *Biochem. J.* **49**, 620.

Further Observations on Histaminase and Diamine Oxidase. By R. Kapeller-Adler. (*Department of Clinical Chemistry, Royal Infirmary, Edinburgh*)

Doubts were previously expressed (Kapeller-Adler, 1944, 1949, 1951) about the identity of histaminase and diamine oxidase, claimed by Zeller (1938*a*,*b*). In the present communication further data are presented suggesting that a distinction should be drawn between histaminase, the enzyme specific for histamine destruction and diamine oxidase, which may inactivate diamines such as cadaverine, putrescine and agmatine.

The effect of placental histaminase and of purified histaminase from pig kidneys on histamine, cadaverine, putrescine and agmatine was investigated in parallel assays using the microvolumetric method for histaminase estimation (Kapeller-Adler, 1951). In agreement with previous results (Kapeller-Adler, 1951, 1952) it was found that kidney extracts were much more active on histamine than on cadaverine, and that placental extracts were more active on cadaverine than on histamine. The effect on putrescine was a very small one in kidney extracts, somewhat larger in placental extracts, but in both instances much smaller than that on histamine. Whereas kidney extracts had no effect on agmatine, a weak but consistent activity was seen with agmatine in placental extracts. A striking difference between histamine and the other substrates was further obtained when kidney or placental extracts were acting on mixtures of these substrates. Whereas cadaverine, putrescine and agmatine did not interfere with the histaminase-histamine reaction, these diamines, when applied in mixtures, greatly influenced their respective enzymic inactivation. The non-interference of these diamines with the inactivation of histamine was confirmed in parallel experiments with Anrep's biological test for histaminase (Anrep, Barsoum & Ibrahim, 1947). This finding does not support Werle's (1941) claim that putrescine completely abolishes the histaminase-histamine reaction, nor does it confirm previous observations (Kapeller-Adler, 1949) where an inhibitory effect of cadaverine on histamine inactivation was obtained with a macromethod.

The recent results show that the effect of kidney and placental extracts on histamine cannot be cor-

related with that of these extracts on cadaverine, putrescine or agmatine. They seem to strengthen the theory that histaminase is the specific enzyme for histamine. Moreover, these results make it even appear doubtful whether diamine oxidase is a single enzyme at all, for it is difficult to explain why placental diamine oxidase should attack cadaverine much more actively than it attacks putrescine or agmatine when the corresponding enzymic effect in kidney extracts is equally low on cadaverine and putrescine and is not shown with agmatine.

REFERENCES

Anrep, G. V., Barsoum, G. S. & Ibrahim, A. (1947). *J. Physiol.* **106**, 379.
Kapeller-Adler, R. (1944). *Biochem. J.* **38**, 270.
Kapeller-Adler, R. (1949). *Biochem. J.* **44**, 70.
Kapeller-Adler, R. (1951). *Biochem. J.* **48**, 99.
Kapeller-Adler, R. (1952). *Biochem. J.* (in the Press).
Werle, E. (1941). *Biochem. Z.* **311**, 270.
Zeller, E. A. (1938*a*). *Helv. chim. Acta*, **21**, 880.
Zeller, E. A. (1938*b*). *Helv. chim. Acta*, **21**, 1645.

The Inhibition of Growth and Cozymase Synthesis in Bacteria by Halogen-substituted Nicotinic Acids. By D. E. Hughes. (*Medical Research Council Unit for Research in Cell Metabolism, Department of Biochemistry, University of Sheffield*)

The inhibition of the growth of *Staphylococcus aureus* by 5-fluoronicotinic acid reported by Hawkins & Roe (1949) has been confirmed, and studies have been extended to the mechanism of its action in *Lactobacillus arabinosus*, *Proteus vulgaris* and *Escherichia coli*. Growth in all four micro-organisms is inhibited by 5-fluoro-, 5-bromo- and 5-chloronicotinic acids, whilst 2- and 6-fluoronicotinic acids do not inhibit growth. The degree of inhibition increases when the concentration of nicotinic acid in the growth medium decreases. As judged by inhibition indices 5-fluoronicotinic acid is from 100- to 1000-fold more effective as an antagonist of nicotinic acid than either the 5-chloro- or 5-bromo-substituted acids. Nicotinamide, nicotinamide riboside, nicotinamide ribotide and cozymase are all less efficient in reversing the inhibition of growth than nicotinic acid itself. This suggests that it is the catalytic activity of cozymase that is inhibited. In contrast, glycolysis by washed suspensions of nicotinic acid deficient cells of *Lb. arabinosus* and *Staph. aureus*, and oxygen uptake by *Proteus vulgaris* are not inhibited by 5-fluoronicotinic acid, but the stimulation of these processes by added nicotinic acid (Hughes & Williamson, 1952) is inhibited. This suggests that in this case 5-fluoronicotinic acid inhibits cozymase synthesis. This is directly shown by the finding that cozymase synthesis from nicotinic acid in washed suspensions of *Lb. arabinosus* and *Staph. aureus* is strongly inhibited, whilst the cozymase uptake by the cell is not inhibited. No marked effect on the rate of cozymase synthesis was found with 5-chloro- and 5-bromonicotinic acids under the conditions tested. The synthesis of cozymase from nicotinamide and nicotinamide riboside (5×10^{-5}M) is inhibited to the same degree as the synthesis from nicotinic acid (60–80 %) by 5-fluoronicotinic acid (5×10^{-4}M), but the inhibition of the synthesis from nicotinamide ribotide is only 10–20 %. These results indicate that inhibition of growth by 5-fluoronicotinic acid is mainly due to a block of the utilization of nicotinic acid for cozymase synthesis.

REFERENCES

Hawkins, G. F. & Roe, A. (1949). *J. org. Chem.* **14**, 328.
Hughes, D. E. & Williamson, D. H. (1952). *Biochem. J.* **51**, 330.

Collagen Formation in Rabbit Skin. By R. D. Harkness and A. Neuberger. (*National Institute for Medical Research, Mill Hill, London, N.W.* 7)

A relatively soluble protein resembling collagen has been described by Orekhovitch, Tustanovsky, Orekhovitch & Plotnikova (1948), who named it 'procollagen'. The present work was undertaken to obtain evidence whether this material was a precursor of collagen. Young growing rabbits (500–1000 g.) were given by intraperitoneal injection glycine labelled with ^{14}C in the methylene group (10 μc./100 g. body wt.) and killed 1, 3, 7 or 25 days afterwards. 'Procollagen' was prepared from the skin by a modification of the method of Orekhovitch *et al.* (1948). The material obtained consisted of

crystal-like needles or fibres together with some amorphous material. Examination with the electron microscope showed fibres with the typical banding of collagen (about 650 A.), while examination of the products of hydrolysis by paper chromatography showed an amino-acid pattern similar to that of collagen. The 'procollagen' was obtained by four successive extractions of skin which it is estimated removed about 85 % of the total 'procollagen' present. The weight of this material was about 20 % of the total skin collagen. The fractions were kept separate and, after further purification and hydrolysis, DNP-glycine was isolated from them by the method of Perrone (1951) for estimation of isotopic activity. DNP-glycine was prepared similarly from the residual collagen of the skin extracted as gelatin. In the 'procollagen', the distribution of specific activity in the fractions changed systematically with time after injection, being highest in the first fractions at 1 and 3 days, more evenly distributed at 7 days and lowest in the first fractions at 25 days. This change in the distribution of activity agreed with the hypothesis that these represented overlapping stages in the formation of collagen, of different maturity, the first fractions being the youngest. Activity in the collagen of the 3-, 7- and 25-day animals agreed in general with this hypothesis, since it was lower than the 'procollagen' at 3 and 7 days, but higher at 25 days. However, the total active material in the collagen at 1 and 3 days was more than would be expected. At 1 day also, specific activity was higher in the collagen than in the last 'procollagen' fractions in two animals. The evidence available is covered by the hypothesis that 'procollagen' is a precursor of a part of skin collagen, which is inhomogeneous.

We are very grateful to Dr C. E. Challice for examination of material with the electron microscope.

REFERENCES

Orekhovitch, V. N., Tustanovsky, A. A., Orekhovitch, K. D. & Plotnikova, N. E. (1948). *Biochimia*, **13**, 55.
Perrone, J. C. (1951). *Nature, Lond.*, **167**, 513.

The Stability of Lipovitellin to Freezing and Drying. By C. H. Lea and J. C. Hawke. (*Low Temperature Station for Research in Biochemistry and Biophysics, University of Cambridge, and Department of Scientific and Industrial Research*)

Since lipid-protein complexes (lipoproteins) are among the less stable constituents of tissues, an investigation of some of the properties of lipovitellin, and of the effects of freezing and drying and of storage in the frozen and dried states, seemed of interest.

Lipovitellin, prepared as previously described (Lea & Hawke, 1951), was almost completely dispersible between pH 6 and 8 in 15 % (w/v) NaCl saturated with ether. Between pH 5·5 and 8·5 practically no lipid could be extracted from the solution by ether. The isoelectric point as determined by electrophoretic mobility or by the effect of NaCl on pH was 5·5–5·6, in contrast to 3·4–3·5 obtained by Young & Phinney (1951) by a solubility method.

When lipovitellin was rapidly frozen and thawed at pH 6·8, its solubility was only slightly impaired, but similar treatment at pH 5·2 caused a marked loss. On storage in the frozen state (pH 6·8), solubility decreased only very slowly at −29°; at −3° the lipoprotein was almost completely insoluble after 3 months.

Freeze-drying had a rather variable effect on the solubility of lipovitellin, but in all cases the loss was higher when drying was carried out at pH 5·2 than at pH 6·8. Approximately 25 % of the lipid present in lipovitellin freeze-dried at pH 6·8 was readily extractable by ether, although on re-wetting the material recovered most of its capacity for withholding the lipid from ether. Freeze-dried lipovitellin stored at 37° in equilibrium with atmospheres of high relative humidity, i.e. at high moisture contents, became insoluble extremely rapidly, much more rapidly in fact than when suspended in water or dispersed in NaCl solution. In a completely dry atmosphere the rate of change was extremely slow, whilst atmospheres of intermediate humidities produced intermediate rates of change. In all cases insolubility developed more rapidly in material adjusted to pH 5·2 before drying than in that adjusted to pH 6·8. Qualitatively the effects of storage in moist and dry atmospheres on the liberation of free lipid were similar to the effects on solubility, the change being great at high humidities and small at low humidities. Quantitatively the parallelism between the two criteria of decomposition was by no means complete.

REFERENCES

Lea, C. H. & Hawke, J. C. (1951). *Biochem. J.* **50**, 67.
Young, E. G. & Phinney, J. I. (1951). *J. biol. Chem.* **193**, 73.

Formation of Monohydroxyindoles from Dihydroxyphenylalanines. By J. HARLEY-MASON and R. I. T. CROMARTIE. (*Chemical Laboratory, University of Cambridge*)

The formation of 5:6-dihydroxyindole by oxidation of 3:4-dihydroxyphenylalanine is well known. We have now found that oxidation of 2:5-dihydroxyphenylalanine leads to 5-hydroxyindole in high yield. This observation is significant in connexion with the biogenesis of 5-hydroxyindole derivatives occurring in nature, since 2:5-dihydroxyphenylpyruvic acid has been established as an intermediate in the metabolism of tyrosine in liver (Knox & Le May-Knox, 1951), and this keto-acid could be transformed into 2:5-dihydroxylphenylalanine by a transamination.

Similar oxidation of 2:3-dihydroxyphenylalanine leads to 7-hydroxyindole, the yield being much higher than that obtained in the original synthesis (Beer, Clarke, Khorana & Robertson, 1948). This hydroxyindole is therefore now available in sufficient quantity to serve as a starting point for a synthesis of 7-hydroxytryptophan which is of interest as a possible intermediate in the formation of hydroxykynurenine from tryptophan.

REFERENCES

Beer, R. J. S., Clarke, K., Khorana, M. G. & Robertson, A. (1948). *J. chem. Soc.* p. 1605.

Knox, W. E. & Le May-Knox, M. (1951). *Biochem. J.* **49**, 686.

Partial Synthesis of an Acid Containing the Entire Carbon Skeleton of a Natural C_{27} Bile Acid. By R. J. BRIDGWATER and G. A. D. HASLEWOOD. (*Guy's Hospital Medical School, London, S.E.* 1)

An acid, $C_{27}H_{46}O_5$, m.p. 172°, was isolated by one of us from the bile of *Alligator mississippiensis* (Haslewood, 1952). This has been converted to the 'stem acid', $C_{27}H_{46}O_2$, m.p. 104–106°, the partial synthesis of which from cholanic acid is now reported. Our colleague, Dr F. H. Brain, suggested a malonic ester type of synthesis, which was applied as follows: Ethyl cholanate (I, $R_1 = Et$) was converted by lithium aluminium hydride reduction into cholan-24-ol (II), which yielded the corresponding iodide (III). Condensation of III with diethyl methylmalonate gave the di-ester (IV, $R_1 = Et$) which was converted into the di-acid (IV, $R_1 = H$). The di-acid on vacuum distillation yielded coprostan-26-oic acid (V) for which the trivial name 'coprostanic acid' is suggested. Coprostanic acid, m.p. 106–108°, is identical with the stem acid prepared from the 'alligator acid' by Kishner-Wolff reduction and hydrolysis of the corresponding dehydro-ethyl ester. Comparison of the two infrared spectra was kindly carried out for us by Dr A. R. H. Cole, on instruments made available to him at the Chester Beatty Institute.

$$\underset{\text{I}}{R.{}^{24}COOR_1} \rightarrow \underset{\text{II}}{R.CH_2OH} \rightarrow \underset{\text{III}}{R.CH_2I}$$

$$\rightarrow \underset{\text{IV}}{R.CH_2.CMe(CO_2R_1)_2} \rightarrow \underset{\text{V}}{R.{}^{24}CH_2CHMeCOOH}.$$

R = norcholanyl.

This is the first occasion on which conclusive proof has been obtained for the carbon skeleton of any bile acid or bile alcohol containing more than 24 carbon atoms.

REFERENCE

Haslewood, G. A. D. (1952). *Biochem. J.* **50**, xxxv.

The Metabolism of *Vibrio desulfuricans* in anaerobic Petroliferous Formations. By K. MACKENZIE. (*Trinidad Leaseholds Ltd., Pointe-a-Pierre, Trinidad*)

The researches of ZoBell (1938) and other workers have established that certain types of anaerobic bacteria are commonly found in the saline waters associated with oil-bearing sands. Carbon is assimilated from the crude oil, which is broken down partly into lighter oil and gas. ZoBell (1943*a*) has shown that this can be done under laboratory conditions and has made practical tests (ZoBell, 1943*b*; cf. Beck, 1947) in certain 'exhausted' oil fields of Pennsylvania. The secondary recovery of oil by such gaseous repressuring was not successful; posssible causes for this failure are given below.

Three forms of *Vibrio desulfuricans* (*Desulfovibrio desulfuricans*) were obtained from different anaerobic sources and cultured in anaerobic media suggested by ZoBell (1946). One was from an oil sand saturated with water; a second was from a hydrogen generator using salt water as a displacement fluid; a third was obtained from a sea-water line which had been undisturbed for several months and contained much ferrous sulphide and bacterial mycelium. These forms were indistinguishable by staining and Gram test, and may well have been identical; they conformed closely to the description of Beijerinck (1895).

After multiplication in an anaerobic sea-water medium with organic carbon substrate, the bacteria were inoculated with a mixture of crude oil and salt water in the absence of air. Slow but profuse growth of mycelium took place at the oil-water interface, some of the oil was lost, and a liberation of gases, CH_4, H_2 and H_2S, was observed. Controls without oil, or with purified paraffin, or with additions of inhibiting agents, showed no appreciable evolution of gas.

Tests were then made in which *V. desulfuricans* was inoculated into an oil-bearing sand core immersed in a saline mixture to which K and Na phosphates had been added to give a pH of approximately 8·0; an E_H of $-0{\cdot}11$ was maintained. A proliferation of the bacterium was accompanied by gaseous evolution (CH_4, H_2 and H_2S), and the extraction of the oil from the used cores, when compared with the samples tested before treatment, showed that some of the oil had been removed from the formation.

In considering these results, which form part of a programme for the study of secondary oil recovery from exhausted formations, the importance of added phosphate in the proliferation and accelerated growth of the organism in telluric saline waters may be considerable. In aerobic experiments, Gross (1947) has shown the stimulating effect of phosphate additions, and phosphate availability, according to Atkins (1923) and Sverdrup (1942), is a limiting factor in the growth of marine organisms. To be successful, experiments with formations demand the presence of available phosphate, and methods of ensuring this are now being tested.

REFERENCES

Atkins, W. R. G. (1923). *J. Mar. biol. Ass. U.K.* **13**, 119.
Beck, J. V. (1947). *Prod. Mon.* **11**, 13.
Beijerinck, W. M. (1895). *Zbl. Bakt.* II, **1**, 49, 104.
Gross, L. (1947). *Nature, Lond.*, **164**, 239.
Sverdrup, H. U. (1942). *The Oceans.* New York: Prentice Hall.
ZoBell, C. E. (1938). *J. sediment. Petrol.* **8**, 10.
ZoBell, C. E. (1943*a*). *Bull. Amer. Assoc. Petrol. Geol.* **27**, 1175.
ZoBell, C. E. (1943*b*). *Petrol. World, Los Angeles*, **40**, 30.
ZoBell, C. E. (1946). *Marine Microbiology.* Waltham, Mass.: Chronica Botanica.

Incorporation of [*carboxy*-^{14}C]-Acetate into Lactose and Glycerol by the Udder of the Lactating Goat. By G. Popják (*National Institute for Medical Research, Mill Hill, London, N.W.* 7), R. F. Glascock and S. J. Folley (*National Institute for Research in Dairying, University of Reading*)

Lactose and glycerol of neutral fats were isolated from the milk of a goat which was injected with 5 mc. of ^{14}C as [*carboxy*-^{14}C]-acetate. This experiment, the primary object of which was the study of the synthesis of milk fatty acids, has already been described (Popják, French & Folley, 1951).

The specific activity:time curves of lactose and glycerol behave exactly as predicted on theoretical grounds by Zilversmit, Entenman & Fishler (1943) for the relationship between the specific activity-time curves of a labelled precursor (in this case lactose), and its immediate reaction product (in this case glycerol). It is suggested that glucose rather than lactose is actually the precursor of glycerol, and that the galactose and glucose units in lactose are in rapid equilibrium, so that the specific activity of lactose at any moment is not measurably different from that of its precursor, glucose. It has been found by French, Popják & Malpress (1952) that the radioactivities of the glucose and galactose units in lactose are identical after the administration of [^{14}C]-glucose to lactating rabbits.

It is known (cf. Wood, 1948) that, after administration of [*carboxy*-^{14}C]-acetate to an animal, the glucose units in liver glycogen contain practically all the label in the 3:4-positions. The metabolism of [3:4-^{14}C]-glucose should yield [1:3-^{14}C]-glycerol. Degradation of the glycerol by periodate oxidation showed that 95 % of the radioactivity of the molecule was in carbon atoms 1 and 3 and only 5 % in carbon atom 2.

REFERENCES

French, T. H., Popják, G. & Malpress, F. H. (1952). *Nature, Lond.*, **169**, 71.

Popják, G., French, T. H. & Folley, S. J. (1951). *Biochem. J.* **48**, 411.

Wood, H. G. (1948). *Cold Spr. Harb. Symp. quant. Biol.* **13**, 201.

Zilversmit, D. B., Entenman, C. & Fishler, M. C. (1943). *J. gen. Physiol.* **26**, 325.

Incorporation of Glycine into Rabbit Plasma Proteins. By A. Dovey, R. C. Holloway and A. S. McFarlane. (*National Institute for Medical Research, Mill Hill, London, N.W.* 7)

Serum samples obtained at half-hourly intervals after a single intravenous injection of α-[^{14}C]glycine were dialysed against an excess of inactive glycine. When equilibrium was established, the free amino-acids of the diffusates were adsorbed on Zeo Karb 225, washed, eluted and combusted to CO_2. Radioactivities of the gas samples are expressed as counts/min./mg. free amino-acid carbon in the original serum, and also as counts/min./mg. free glycine, on the assumption that all the activity is in the latter.

The proteins were dialysed free of glycine and fractionated with sodium sulphate, the albumin fraction containing also the α- and β-globulins. The fractions were combusted to CO_2 and the radioactivities expressed as counts/min./mg. protein-bound glycine, again on the assumption that glycine is the only labelled amino-acid present. The curves show that the fall in free glycine activity—to less than 10% of the peak value in 2 hr.—is more rapid than was generally believed. At 15 hr., when the protein activities are maximal, the free glycine activity is of the same order as the protein-bound glycine activities. Since new labelled protein is necessarily highly diluted with inactive protein already in the plasma, the relatively high activities observed in the proteins can only be explained if all their labelled glycine was derived from free plasma glycine of high specific activity, i.e. within 2 hr. of the injection. This indicates that the liver acts extremely rapidly in converting dietary amino-acids into plasma protein and is in keeping with recent findings of Miller, Bly, Watson & Bale (1951).

REFERENCE

Miller, L. L., Bly, C. G., Watson, M. L. & Bale, W. F. (1951). *J. exp. Med.* **94**, 431.

Picric Acid as a Reagent for the Isolation and Estimation of Hexone Bases in Protein Hydrolysates. By W. Robson and A. S. M. Selim. (*Biochemical Laboratory, King's College, London*)

Continuous Direct Photometry of Proteins Separated by Electrophoresis in Filter Paper. By E. M. Crook, H. Harris and F. L. Warren. (*Department of Biochemistry, University College, London*)

The estimation of protein fractions after their separation by electrophoresis in filter paper and subsequent staining may be carried out by excision, elution, and colorimetric estimation (Cremer & Tiselius, 1950) or by direct photometry, either intermittent (Grassmann, Hannig & Knedel, 1951) or continuous. The latter methods have the advantage of being less laborious and also of requiring less material. Although certain problems are common to both elution and direct photometric methods (e.g. the problem of equivalent dye-absorption by different protein fractions), special problems arise in direct photometry. These include (i) reduction of light scatter in the paper; (ii) necessity for an efficient optical system; (iii) calibration of the instrument in terms of the amount of dye on the paper.

The present communication describes an apparatus and a technique for direct continuous photometry. Electrophoresis is carried out by a method essentially similar to those already published (e.g. Flynn & de Mayo, 1951). The dried strips are dyed with azocarmine-B (Plückthun & Götting, 1951) and redried. The paper is rendered translucent by immersion in a fluid of approximately the same refractive index as cellulose (Grassmann *et al.* 1951). The soaked strips are laid on glass plates and covered with cellophan which is pressed

into close contact to eliminate air bubbles. The strips are then transferred to a trolley which can be moved along a track by a synchronous electric motor in such a way that the whole length of the paper strip passes between an illuminated slit and a photocell. The current from the photocell is amplified and recorded on a pen-recorder. Successive coloured bands of protein are indicated by decreases of photocell current.

The relation between current and intensity of transmitted light is determined by substituting an optical wedge for the coloured paper and recording the current over the density range of the wedge. The instrument behaves linearly in this respect.

Calibration of the current in terms of the amount of dye on the paper is necessary because, owing to the wide range of density between different coloured bands and to the inhomogeneous nature of the light absorbing medium, it cannot be assumed that Beer's law is applicable. Calibration was achieved by constructing a coloured 'optical wedge' from strips of filter paper soaked in serial dilutions of human plasma and dyed with azocarmine. The quantity of dye in each 'step' of this wedge was determined independently by elution and colorimetry on duplicate strips of the same area. This calibration showed that the relation between current and quantity of dye actually on the paper is hyperbolic over the whole range and not logarithmic.

REFERENCES

Cremer, H. D. & Tiselius, A. (1950). *Biochem. Z.* **320**, 273.

Flynn, F. V. & de Mayo, P. (1951). *Lancet*, **2**, 235.

Grassmann, W., Hannig, K. & Knedel, M. (1951). *Dtsch. med. Wschr.* **76**, 333.

Plückthun, H. & Götting, H. (1951). *Klin. Wschr.* **29**, 415.

DEMONSTRATIONS

Technique of Gas Counting of $^{14}CO_2$. By R. C. Holloway and A. S. McFarlane. (*National Institute for Medical Research, Mill Hill, London, N.W.* 7)

Gas-liquid Partition Chromatography Applied to Volatile Bases. By A. T. James. (*National Institute for Medical Research, Mill Hill, London, N.W.* 7)

Preparation of Conductivity Water. By S. Jacobs (introduced by A. Neuberger). (*National Institute for Medical Research, Mill Hill, London, N.W.* 7)

The Chromatographic Separation and Simultaneous Estimation of Naturally Occurring Substances Containing ^{131}I. By J. Gross* and A. J. P. Martin. (*National Institute for Medical Research, Mill Hill, London, N.W.* 7)

A kieselguhr column loaded with 0·5 N-NaOH (see Grönkvist & Hellberg, 1951) is used with solvents ranging from butanol + 20 % $CHCl_3$ to pure butanol for separating thyroxine, triiodothyronine, iodide, diiodotyrosine and monoiodotyrosine. The effluent from the column is passed through a Geiger counter to a fraction cutter. The radioactivity is recorded continuously by means of a count-rate meter and recording milliammeter; the movements of the fraction cutter are marked on the same chart.

* Merck Fellow in the biological sciences of the National Research Council of Canada.

REFERENCE

Grönkvist, K. E. & Hellberg, H. (1951). *Farm. Revy*, **50**, 189.

Methods for the Examination of Urinary Metabolites of Tryptophan. By C. E. DALGLIESH. (*National Institute for Medical Research, Mill Hill, London, N.W.* 7)

The group isolation of the metabolites from urine, and the diagnostic reactions by which they may be identified, will be demonstrated.

A Mechanical Cutter for Preparing Small Tissue Specimens with Minimal Disturbance of Cell Structure. By H. MCILWAIN and H. L. BUDDLE. (*Biochemical Laboratories, Institute of Psychiatry, Maudsley Hospital, S.E.* 5)

The apparatus cuts by passing a vertical blade into the specimen, which is placed on an intermittently moving table. It was devised to prepare pieces of animal tissues for metabolic experiments and to be especially applicable to small and irregular specimens available at biopsy or from small organs. Its special features are to cause much less disturbance of cell structure than homogenizers or blenders, and to be applicable to fragments which would be difficult or impossible to slice by ordinary methods.

Structural elements which are disrupted on homogenizing exert a controlling influence on many metabolic characteristics: on levels of tissue constituents or of metabolic rates; on the concentration or exclusion of added substances; and on the response of tissues to inhibiting and stimulating agents. Slices of 0·2 mm. or less in thickness, and prisms $0{\cdot}2 \times 0{\cdot}2$ mm. or less in cross-section, can be prepared with the present cutter in 30 sec. It has been applied to liver, kidney and various parts of the central nervous system. Suspensions of guinea pig, rat and human cerebral cortex as prisms $0{\cdot}2 \times 0{\cdot}2 \times 2$ mm. can be pipetted. They respired in simple glucose-phosphate salines at rates within 5% of those of slices cut in the normal way, and double those of homogenates in the same medium and in the same tissue concentration (20 mg./ml.). Respiration by the chopped tissue was stable for over 2 hr. during which the rate in homogenates fell considerably. Aerobic formation of lactic acid by the chopped tissue approached values for slices.

The rates at which the mechanically cut tissue respired and formed lactic acid aerobically, were doubled or trebled by the electric and metabolic conditions which induce such changes in sliced, but not in homogenized, cerebral cortex.

Some (but not all) manually made breis, minces and scissor-cut tissues have probably approached the condition of the tissue described here, but their use has greatly decreased with the advent of blenders and homogenizers. These instruments have often been applied to problems which would have been tackled better with preparations in which cell structure was retained, and for such purposes, the present machine gives from many specimens more defined preparations than can be made by hand.

Some Improvements and Precautions in Methods for the Paper Chromatography of Steroids. By I. E. BUSH (introduced by R. K. CALLOW). (*National Institute for Medical Research, Mill Hill, London, N.W.* 7)

[*The Editors of the Biochemical Journal accept no responsibility for the Reports of the Proceedings of the Society. Abstracts of papers read are published as received from the authors.*]

PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **307th Meeting** *of the Biochemical Society was held in the Department of Biochemistry, University of Liverpool, Liverpool 3, on Friday, 18 April 1952 when the following papers were read.*

COMMUNICATIONS

A Vitamin B_{12}-binding Factor in Sow's Milk. By MARGARET E. GREGORY, J. E. FORD and S. K. KON. (*National Institute for Research in Dairying, University of Reading*)

A sample of cow's milk, prepared for assay by warming in the presence of a trace of sodium cyanide, contained 0·004 μg. vitamin B_{12}/ml. by the *Lactobacillus leichmannii* 4797 assay. Vitamin B_{12} added to the milk before extraction was quantitatively recovered. However, *Lb. leichmannii* did not respond to a similar extract of sow's milk or to vitamin B_{12} added to the extract up to a concentration of 0·05 μg./ml. whole milk, but responded to additions over and above that amount.

Vitamin B_{12} was largely (92 %) recovered in the ultrafiltrate from cow's milk to which 0·04 μg. vitamin B_{12}/ml. had been added, whereas none could be detected in the ultrafiltrate from sow's milk similarly treated. Furthermore, vitamin B_{12}, added at a rate of 0·05 μg./ml. whole milk, could not be dialysed from sow's milk, but 90 % dialysed from cow's milk. Therefore, it appears that vitamin B_{12}, added to sow's milk, is bound and microbiologically inactivated. This behaviour of sow's milk is similar to that reported by Bird & Hoevet (1951) for an intrinsic factor concentrate. Since the vitamin B_{12} naturally present in cow's milk was not ultrafiltrable, it must also be 'bound', but in a form which, after being heated with the assay medium, is available to micro-organisms.

The vitamin B_{12}-binding component of sow's milk survived heating at 100° for 30 min. It was destroyed by autoclaving at pH 12, but not at pH 7 unless a trace of sodium cyanide was present. It was not ultrafiltrable and was inactivated by papain digestion.

Fractionation of the soluble whey proteins with ammonium sulphate, according to the procedure of Prusoff, Meacham, Heinle & Welch (1950) for the isolation of the intrinsic factor from powdered hog stomach, indicated that the vitamin B_{12}-binding activity was associated with the fraction precipitated by 35–55 % ammonium sulphate. Sow's milk casein had no binding activity.

Further work on the isolation and characterization of this binding factor is in progress.

We wish to thank Dr R. Braude and Mr K. G. Mitchell for samples of sow's milk.

REFERENCES

Bird, O. D. & Hoevet, B. (1951). *J. biol. Chem.* **190**, 181.
Prusoff, W. H., Meacham, G. C., Heinle, R. W. & Welch, A. D. (1950). *Abstr. Pap. Amer. chem. Soc.* 118th Mtg, p. 27A.

Determination of Sulphate in the Study of Sulphatases. By K. S. DODGSON and B. SPENCER. (*Physiology Institute, Newport Road, Cardiff*)

Studies on sulphatases have been hindered in the past by the lack of suitable methods for the determination of small amounts of sulphate under varying experimental conditions. It appeared to us that the micro-benzidine method might be adapted for this purpose. Various modifications of this method have been described (e.g. Tanaka, 1938; Power & Wakefield, 1938; Eichelberger, Brower & Roma, 1951), but all demand rigid adherence to the prescribed conditions. Considerable research has been necessary in order to establish techniques suitable for enzyme studies.

Briefly, the incubated enzyme-substrate mixture is deproteinized with ethanol, the acidity of the protein-free solution is adjusted to within certain critical limits with trichloroacetic acid, and sulphate is precipitated as benzidine sulphate with an ethanolic solution of purified benzidine. The benzidine sulphate is separated and determined colorimetrically after diazotization and coupling with thymol. In this and other modifications which we have tested, sulphate is not precipitated quantitatively in amounts less than 10 μg., consequently enzyme control determinations may be low. In

practice this difficulty is met by ensuring that the amount of sulphate in the control is always of the order of 15 μg. In experiments with marine organisms this amount of sulphate is normally encountered, but with mammalian tissues it is necessary to add sulphate (preferably in the trichloroacetic acid) to tests and controls immediately before addition of benzidine.

With this method we can estimate differences between enzyme test and control of 10–120 μg. with an accuracy of $\pm 5\%$; while the method is useful for measuring even smaller differences. Added sulphate can be recovered quantitatively from various tissue homogenates of the rat, cat, dog and many marine organisms and seaweeds after incubation periods of up to 3 hr. and in the presence of acetate buffers of varying molarity and pH. Recoveries are not affected by K, Na, Mg, Cl, F, CN and PO_4 ions at concentrations of 0·01 M, but results are low in the presence of similar concentrations of Ca, Ba and Fe.

We have checked this method against the spectrophotometric assay method of Dodgson, Spencer & Thomas (1952) and find that the arylsulphatase of various rat organs releases *p*-hydroxyacetophenone and inorganic sulphate from potassium *p*-acetylphenylsulphate in molecular proportions.

REFERENCES

Dodgson, K. S., Spencer, B. & Thomas, J. (1952). *Biochem. J.* **51**, i.

Eichelberger, L., Brower, T. D. & Roma, M. (1951). *Amer. J. Physiol.* **166**, 328.

Power, M. H. & Wakefield, E. G. (1938). *J. biol. Chem.* **123**, 665.

Tanaka, S. (1938). *J. Biochem., Tokyo*, **28**, 37.

The Action of Crystalline β-Amylase on Some Glycogens. By D. J. MANNERS. (*Biochemical Laboratory, University of Cambridge*)

β-Amylolysis of amylopectins and glycogens is now generally regarded as a stepwise hydrolytic liberation of maltose from the exterior chains. The amount of maltose thus formed is determined by the position of the branching point of the unit chain, and hence by the exterior chain length.

Glycogens from liver (rabbit, foetal sheep, cat), muscle (rabbit, horse, man), and invertebrates (*Mytilus edulis* I, *Ascaris lumbricoides*) of unit chain lengths 11–13 radicals (potassium periodate method, Halsall, Hirst & Jones (1947) as modified by Bell & Manners, 1952) were treated to completion with crystalline sweet potato β-amylase, kindly supplied by Prof. C. S. Hanes, F.R.S. The resulting conversions to maltose (41–49 %) corresponded to β-limit dextrins of chain lengths 6–7.

Three other glycogens have been examined: (*a*) *Mytilus edulis* II (unit chain length 16 by periodate, and 18 by methylation (Bell, 1936)) gave 47 % conversion to maltose corresponding to a β-limit dextrin of 9–10 glucose radicals; (*b*) *Mytilus edulis* III (unit chain length approx. 5 by periodate, cf. Halsall *et al.* 1947) gave 43 % conversion to maltose, corresponding to a β-limit dextrin of three glucose radicals; (*c*) *Helix pomatia* (unit chain length 7 by periodate; cf. Baldwin & Bell (1940), who found a chain length of 12, by methylation, for a different sample) gave 37 % conversion to maltose, corresponding to a β-limit dextrin of 4 glucose radicals. Control experiments showed that the β-amylase was free from maltase, α-amylase and *Z*-enzyme.

It is interesting to compare the above β-amylolysis of 12-unit glycogens with those of an 18-unit rabbit-liver glycogen. Halsall, Hirst, Hough & Jones (1949) found that this material gave 53 % maltose on β-amylolysis, and a β-limit dextrin of 9 glucose radicals. Amylopectins (unit chain length 19–26, cf. Bell, 1947) give β-amylolysis limits of 50–62 %, and β-limit dextrins of chain length 11–13.

The present study supports, chemically, the suggestion of Barker, Bourne & Stacey (1950) 'that there is no clear-cut distinction in nature between glycogen and amylopectin'. Between the extremes, 12-unit glycogens and 25-unit amylopectins, lie a group of polysaccharides of intermediate properties, including 18-unit glycogens, and certain bacterial and protozoal polysaccharides.

I wish to thank Dr D. J. Bell for the glycogen samples, and for valuable advice and encouragement.

REFERENCES

Baldwin, E. & Bell, D. J. (1940). *Biochem. J.* **34**, 139

Barker, S. A., Bourne, E. J. & Stacey, M. (1950). *J. chem. Soc.* p. 2884.

Bell, D. J. (1936). *Biochem. J.* **30**, 2144.

Bell, D. J. (1947). *Ann. Rep. Chem. Soc.* **44**, 217.

Bell, D. J. & Manners, D. J. (1952). *J. chem. Soc.* (in the Press).

Halsall, T. G., Hirst, E. L., Hough, L. & Jones, J. K. N. (1949). *J. chem. Soc.* p. 3200.

Halsall, T. G., Hirst, E. L. & Jones, J. K. N. (1947). *J. chem. Soc.* p. 1399.

The Metabolism of [1-^{14}C]-Benzene. By D. V. PARKE and R. T. WILLIAMS. (*Department of Biochemistry, St Mary's Hospital Medical School, London, W. 2*)

Previous work from this laboratory on the metabolism of benzene in the rabbit had shown that about 60% of the benzene fed could be accounted for in various ways. The acquisition of benzene containing one carbon atom marked with ^{14}C has now enabled us to account for most of the dose of benzene administered and to estimate the amounts of the various metabolites excreted. Thus in a rabbit which had received 1 g. of benzene containing 0·1 mc. of radioactivity, 46% of the benzene appeared in the expired air as benzene in 30 hr., 35% in the urine as metabolites and 16% remained in the tissues as metabolites after 2 days (total, 97%). In this particular experiment the percentage of the dose excreted as various metabolites in 2 days, determined by isotope dilution, was as follows: phenol, 16·7; quinol, 6·2; catechol, 1·2; hydroxyquinol, 0·35; *trans-trans*-muconic acid, 1·8; phenylmercapturic acid, 0·7%. By the same technique it was found that the following substances were not urinary metabolites of benzene: resorcinol, pyrogallol, diphenyl, *p*-diphenylylglucuronide, *cis-cis*- and *cis-trans*-muconic acids, and oxalic, succinic, adipic and acetic acids. In another experiment with 0·88 g. benzene containing 4 μc. of ^{14}C, radioactive metabolites were being excreted for 7 days after dosing, 29·7% of the dose appearing on the first day and 0·4% on the seventh day. In this case 41·3% of the benzene was excreted in the urine as metabolites in 7 days, and 34% in the expired air in 2 days. A 2-day urine in this case contained phenol, 23·7; quinol, 4·8; catechol, 2·1; hydroxyquinol, nil; *trans-trans*-muconic acid, 1·0% of the dose. These items account for 31·6% of the dose in 2 days, and this compares well with the figure 34·8% as the total radioactivity of the 2-day urine.

These and other results were discussed.

A Visual Matching Technique for Paper Electrophoresis of Serum Proteins. By A. L. LATNER and M. W. RICHARDSON. (*Department of Pathology, Medical School, King's College, Newcastle-upon-Tyne*)

This method is suitable for most clinical purposes and requires no photoelectric scanning device.

A pencil line is drawn perpendicular to the long edge across a strip of Whatman no. 1 filter paper 37 × 2 cm. at a distance of 2 cm. from the mid-line. On either side of this original line and 2 mm. from it two further lines are drawn.

Using a micro-pipette or brush, serum is applied along the central line at such a rate that it just spreads to the outer lines. In this way a band of serum 4 mm. wide is obtained across the paper.

The process is repeated on four other strips of paper using 8/10, 6/10, 4/10 and 2/10 dilutions of the serum in normal saline. The serum bands are dried, separated electrophoretically and subsequently stained together according to the technique already demonstrated by one of us (Latner, 1952), although the apparatus used was that of Flynn & de Mayo (1951). The albumin is made to pass down the paper and not over the glass rod.

The albumin bands were originally given numbers 10, 8, 6, 4 and 2, according to the serum dilution. The stained protein bands on the strip made with whole serum are visually matched against each albumin band on the other strips using Ilford Filter no. 204. A number is thus assigned to each of the bands of the whole serum and the percentage of each is calculated:

$$\% = \frac{\text{Band number} \times 100}{\text{Sum of band numbers}}.$$

The band of γ-globulin has approximately twice the other band widths. It is therefore read as if composed of two bands.

It soon became obvious that the spread of a protein solution such as serum is dependent on the protein concentration. This meant that the albumin bands were not in the proportion 10, 8, 6, 4 and 2 and corrections had to be applied. These were determined by studying the area of spread of fixed volumes of varying dilutions of sera on filter paper. It was thus demonstrated that the actual albumin numbers should read 10, 7·5, 5·2, 3·2 and 1·5.

The method has been checked by using protein solutions of known composition. Good duplication has been obtained with repeated samples of sera.

REFERENCES

Flynn, F. V. & de Mayo, P. (1951). *Lancet*, **2**, 238.
Latner, A. L. (1952). *Biochem. J.* **51**, xii.

Non-Protein Nitrogen Fractions of the Flesh of Lobsters and Crabs. By DOROTHY FRASER, W. O. KERMACK, H. LEES and J. D. WOOD. (*Department of Biological Chemistry, Aberdeen University*)

The muscles of the lobster (*Homarus vulgaris*) and the crab (*Cancer pagurus*) have been subjected to 16 hr. extraction with 80 % ethanol at room temperature. The extracts, after clarification by centrifuging, were analysed for total nitrogen, amino nitrogen (Pope & Stevens, 1939), trimethylamine oxide, volatile bases, creatine and creatinine. Two-dimensional paper chromatograms (phenol/collidine) were run to identify the amino-acids present. The amino-acids were estimated by a procedure similar to that described by Fowden (1951), except that the intensity of the ninhydrin colour was determined on a Unicam diffraction-grating spectrophotometer at a wavelength corresponding to the absorption peak of the particular amino-acid being estimated.

Of the total nitrogen present in the extracts we have accounted for about 80 %, made up as follows: 60 % as amino-acid and amide nitrogen, 5 % as volatile bases, and 15 % as trimethylamine oxide. Creatine and creatinine were present at most only in traces. The principal amino-acids in both species were proline, glycine and taurine, together with glutamine and asparagine. Certain other amino-acids were present in small amounts, but no polypeptides have been detected. In so far as our results are comparable with those of Camien, Sarlet, Duchateau & Florkin (1951), the qualitative agreement is good. Lobster blood was examined and found to contain only small amounts of soluble nitrogen compounds.

When lobster muscle was kept at room temperature for 24 hr. either in air or under nitrogen, and the product then extracted as just described, the total nitrogen content of extracts was considerably increased, the increase being located in the amino-acid and volatile base fractions, while the amount of trimethylamine oxide fell. Most of the glutamine appeared to have been hydrolysed to glutamic acid. On the other hand, when the lobster flesh was kept for 72 hr. at 0°, these post-mortem changes did not occur.

REFERENCES

Camien, M. N., Sarlet, H., Duchateau, G. & Florkin, M. (1951). *J. biol. Chem.* **193**, 881.

Fowden, L. (1951). *Biochem. J.* **48**, 327.

Pope, C. G. & Stevens, M. F. (1939). *Biochem. J.* **33**, 1070.

A New Aspect of Ethanol Metabolism: Isolation of Ethyl-glucuronide. By I. A. KAMIL, J. N. SMITH and R. T. WILLIAMS. (*Department of Biochemistry, St Mary's Hospital Medical School, London, W. 2*)

Bartlett & Barnet (1949) have shown that ethanol (1 g./kg.) marked with ^{14}C is largely oxidized to CO_2 in rats, 90 % being so oxidized and eliminated in 10 hr. Both carbon atoms yield CO_2 (Dontcheff, 1950). Neubauer (1901) suggested that it formed a glucuronide in rabbits, but Deichmann & Thomas (1943) have stated that 3·5 ml./kg. of ethanol do not affect the glucuronide output of rabbits.

We have now been able to prove that in rabbits ethanol is excreted to a minor extent as a glucuronide, the amount of conjugation rising with increasing dose. At 1 ml./kg. the conjugation was 0·5 % of the dose, at 2·4 ml./kg. 0·7 %, and at 4·6 ml./kg. 1·6 %, as estimated by the naphthoresorcinol method. (These doses are well below the toxic dose which is about 10–12 ml./kg.) That the colorimetric method was estimating glucuronic acid was confirmed by the isolation of β-ethyl-D-glucuronide from the urine, as the *triacetyl methyl ester*, m.p. 144° and $[\alpha]_D^{20°}$ –32·8 ($CHCl_3$). (Found: C, 49·75; H, 6·4. $C_{15}H_{22}O_{10}$ requires C, 49·7; H, 6·1 %.) It is well known that ethanol is rapidly oxidized *in vivo*, but the present results suggest that very large doses may partly overwhelm the oxidation mechanism and thus allow conjugation to take place.

The excretion of increased glucuronide after ethanol only lasts for 1 day after dosing. Preliminary results with methanol suggest that this alcohol also forms a glucuronide, but this has not been proved by isolation. After methanol there is a slight rise in apparent glucuronide output which lasts several days after dosing. Lund (1948) has shown that methanol is slowly oxidized and excreted compared with ethanol.

REFERENCES

Bartlett, G. R. & Barnet, H. N. (1949). *Quart. J. Stud. Alcohol*, **10**, 381.
Deichmann, W. B. & Thomas, G. (1943). *J. industr. Hyg.* **25**, 286.
Dontcheff, L. (1950). *C.R. Acad. Sci., Paris*, **231**, 177.
Lund, A. (1948). *Acta Pharmacol. toxicol.* **4**, 99, 108.
Neubauer, O. (1901). *Arch. exp. Path. Pharmak.* **46**, 133.

Carotene Synthesis by some Naturally occurring Mutants of *Phycomyces blakesleeanus* and by *Phycomyces nitens*: Inhibition of Carotenogenesis by Streptomycin. By T. W. Goodwin and L. A. Griffiths. (*Department of Biochemistry, University of Liverpool*)

Phycomyces blakesleeanus var. *piloboloides* (+ and −strains), *P. blakesleeanus* mut. *gracilis* (+), *P. blakesleeanus* mut. *pallens* (−) and *P. nitens* (+) were examined qualitatively and quantitatively for polyenes. All produced the same polyenes, in the same relative amounts, as the parent strain (Goodwin, 1952). *Piloboloides* (−) and *pallens* (−) synthesize at the same rate the same absolute amount of β-carotene as the (−) strain of *P. blakesleeanus* (Garton, Goodwin & Lijinsky, 1951). *Piloboloides* (+) and *nitens* (+) synthesize the same amount as *P. blakesleeanus* (+). *Gracilis* (+), however, synthesizes as much as *P. blakesleeanus* (−); furthermore, during the early stages of growth synthesis occurs in the mutant at a much greater rate than in the parent. Dry wt. production by *P. nitens* (+) is only 60 % of that of *P. blakesleeanus* whilst that of all the others is the same. Lipid synthesis is very similar in all the organisms.

Streptomycin inhibits carotenogenesis in *Phycomyces* growing under normal conditions in the light, the degree of inhibition varying linearly with the concentration up to 0·05–0·075 % (w/v). Inhibition at this level is 60–70 %. Further increase in concentration of streptomycin up to 1·6 % (w/v) does not result in further inhibition. Similar inhibition is observed when mycelia are grown in the dark and when well developed mycelia are transferred to streptomycin-containing media. No effect on dry-weight production or lipogenesis was observed at any concentration, although growth rate is always reduced slightly in the early stages. All the polyenes present are affected equally by streptomycin, in contrast to the effect observed with diphenylamine (Goodwin, 1952).

As it is never possible, even with large concentrations of streptomycin, to reduce the carotene levels below about 40 % of the normal, it seems likely that the immediate precursors of carotene can be synthesized by two independent routes, one of which is unaffected by streptomycin. This possibility is being actively investigated.

REFERENCES

Garton, G. A., Goodwin, T. W. & Lijinsky, W. (1951). *Biochem. J.* **48**, 154.
Goodwin, T. W. (1952). *Biochem. J.* **50**, 550.

Action Pattern of Potato Phosphorylase. By J. M. Bailey and W. J. Whelan. (*Chemistry Department, University College of North Wales, Bangor*)

It has been assumed, but never proved, that the synthesis of amylose chains by potato phosphorylase occurs by the simultaneous apposition of glucose units (from glucose-1-phosphate) to the primer molecules (multi-chain hypothesis), as opposed to the successive synthesis of molecules of high molecular weight (single-chain hypothesis) as suggested by Bernfeld & Meutémédian (1948). If the assumption of multi-chain action were correct then synthesis by phosphorylase with a homogeneous primer would afford a homogeneous linear polysaccharide of known chain length. Such homogeneous primers have been prepared by Bailey, Whelan & Peat (1950), and the following observations confirm that synthesis with phosphorylase proceeds by a multi-chain mechanism. (1) With maltohexaose as primer in the polysaccharide synthesis, the wavelength of peak absorption ($\lambda_{max.}$) of the iodine-stained products increases gradually with time of incubation from 490 mμ. (chain length, 12 units calculated from phosphate liberation) to 645 mμ. (chain length > 450 units), corresponding with colour changes from brown through red and purple to blue. During phosphorolysis of the final product of synthesis, the shift in peak absorption was the exact reverse of that

observed in synthesis. (2) Increase in the concentration of the primer (at the same glucose-1-phosphate concentration) results in a corresponding shift in $\lambda_{max.}$ of the iodine stain of the final product. (3) The action pattern is not altered by changes of pH or temperature, both of which affect the mode of action of β-amylase, causing it to vary between a multi-chain and a single-chain mechanism (French, Knapp & Pazur, 1950; Bailey & Whelan, unpublished results). It should be mentioned that by electrokinetic ultrafiltration analysis (Mould & Synge, 1931) of three of our polysaccharide preparations of average chain lengths 33, 77 and 145 units, synthesized with maltohexaose as primer, Drs D. L. Mould and R. L. M. Synge have found that the preparations each have a fairly compact distribution of molecular weights around the average and are readily resolved from one another.

REFERENCES

Bailey, J. M., Whelan, W. J. & Peat, S. (1950). *J. chem. Soc.* p. 3692.

Bernfeld, P. & Meutémédian, A. (1948). *Helv. chim. Acta*, **31**, 1735.

French, D., Knapp, D. W. & Pazur, J. H. (1950). *J. Amer. chem. Soc.* **72**, 1866.

Mould, D. L. & Synge, R. L. M. (1951). *Biochem. J.* **50**, xi.

The Minimum Chain Length for *Q*-Enzyme Action. By J. M. Bailey, S. Peat and W. J. Whelan. (*Chemistry Department, University College of North Wales, Bangor*)

Hobson, Whelan & Peat (1951) have indicated that the ability of *Q*-enzyme to form a ramified amylopectin-like polysaccharide from unbranched amylose-type chains is dependent upon the chain length of the substrate. For example, the enzyme failed to act on an amylose α-dextrin of average chain length 25 units. The observation is consistent with the view that the synthesis of α-1:6-branch linkages must be preceded by the scission of α-1:4-linkages. The purpose of the present investigation was to determine the minimum length of amylose chain which could serve as a substrate for *Q*-enzyme. The proven multi-chain action of phosphorylase (see preceding communication) makes possible the synthesis of homogeneous substrates of known chain length.

A digest containing potato phosphorylase, *Q*-enzyme, glucose-1-phosphate, maltohexaose (primer), and ammonium molybdate (phosphatase inhibitor, see Bailey, Thomas & Whelan, 1951), and a control digest from which *Q*-enzyme was omitted were incubated at 35°. Portions of each were withdrawn at intervals for the estimation of phosphate and intensity of iodine stain (absorption value 680 mμ.). The chain length of the synthetic polysaccharide at any given time was calculated from the mineral phosphate liberated. It was observed that *Q*-enzyme was without influence on the absorption value (680 mμ.) until the linear chains had attained a length greater than 42 units. In a second similar experiment portions of the digest were removed and treated with pure β-amylase (from soya bean) at pH 3·6. The β-amylolysis of the synthetic polysaccharides was complete only if the chain length were less than 40 units. With chains of greater length, branching occurred and the product was not completely hydrolysed by β-amylase. Finally, *Q*-enzyme was allowed to act on two linear polysaccharides (synthesized by phosphorylase) of chain length 58 and 30 respectively. *Q*-enzyme had a very slow action on the 30-unit chain, but branching of the 58-unit chain occurred at 20–25 times as great a rate.

REFERENCES

Bailey, J. M., Thomas, G. J. & Whelan, W. J. (1951). *Biochem. J.* **49**, lvi.

Hobson, P. N., Whelan, W. J. & Peat, S. (1951). *J. chem. Soc.* p. 596.

Increase in Liver Weight and some other Metabolic Effects Produced by Administration of ACTH to Young Guinea Pigs. 1. By M. N. Bland, B. J. Constable, L. J. Harris and R. E. Hughes. (*Dunn Nutritional Laboratory, University of Cambridge and Medical Research Council*)

The original objective in these experiments was to ascertain whether the fall in concentration of ascorbic acid in the suprarenals (and possibly in the liver, and other tissues), which is known to follow the administration of ACTH, might, if continued long enough, ultimately cause a sufficient drainage of the vitamin from the body, as to increase the requirement for the vitamin, and so to hasten the onset of scurvy in deficient animals. Although the answers to the questions posed were mostly negative, some unexpected findings emerged in the course of the trials: in particular, remarkable increases were observed in the weights of the livers of the young growing guinea pigs given repeated doses of ACTH.

In a typical later experiment, guinea pigs were kept on a scorbutogenic basal ration (Sussex ground oats 65·0, bran 14·4, dried yeast 10·8, dried egg yolk 8·0, salt mixture 1·8; halibut-liver oil, 3 drops per animal per week) supplemented with 25 mg. daily of vitamin C. Intramuscular injections of 8 mg./day of ACTH (Armour) were given, in divided doses (4 times/day). Groups of the animals were killed and examined at 2-day intervals. After 10 days, the weights of the livers of the animals treated with ACTH ranged from 7·35 to 8·05 (as % of the body weight), compared with 4·95 to 5·35 for the controls given dummy injections for 10 days, and 4·31 to 5·51 for controls at the start of injection. The ACTH at the levels administered did not depress the normal gains in body weight.

This effect of ACTH in increasing liver weight was already apparent after only 2 days of dosing, and continued thereafter, day by day. The effect has been repeatedly observed in a series of six separate trials, extending over 8 months, in which a total of over 250 guinea pigs have been tested; and it is of a very high order of statistical significance.

The application of these findings to the construction of dose-response curves, comparing the effects of cortisone and ACTH fractions in quantitative biological assays, on guinea pigs and other species, will be discussed.

Increase in Liver Weight and some other Metabolic Effects Produced by Administration of ACTH to Young Guinea Pigs. 2. By R. E. Hughes, L. J. Harris, B. J. Constable and M. N. Bland. (*Dunn Nutritional Laboratory, University of Cambridge and Medical Research Council*)

Under the conditions described in the preceding abstract, the injection of ACTH caused some decline in the concentration of ascorbic acid in the guinea pigs' livers, and also in their adrenals (which likewise increased progressively in weight, e.g. by +75% after 10 days in one typical experiment); such findings were to be expected from results recorded in earlier literature. However, it was found that the total quantities of ascorbic acid in the two organs did not decrease, but were sometimes appreciably higher even than in the controls. No important effect was observed on the concentration of glutathione in the liver.

The concentration of glycogen in the livers of the animals treated with ACTH was several times higher than in the controls (in agreement with earlier reports), but this rise was insufficient in itself to account for the augmentation in liver weight induced by ACTH. (Thus in one typical experiment, the average weights of the livers, and the average concentrations therein of fat, water, protein (N × 6·25) and glycogen were 27·2 g., and 9·1, 64·9, 17 and 4·8%, respectively, after 10 days of injection with ACTH, as compared with 17·9 g., and 11·53, 64·9, 22·6 and 2·13%, respectively, for controls given dummy injections for 10 days, and as compared with 16·1 g., and 7·35, 67·8, 19·8 and 2·16%, for comparable controls at the start of injection.)

In some further parallel experiments, young growing guinea pigs were kept on a natural, mixed diet (bran, oats, cabbage, mangold, hay, halibut liver oil), in place of the basal ration, and the results have been generally similar. On this diet the liver fat was much lower than on the basal ration containing egg yolk and yeast. In the conditions of this trial, on the natural diet, however, an unexpected but consistent effect of ACTH was to decrease the fat content of the liver (e.g. from average 2·12% for dummy-injected controls, to average 0·83% for ACTH-injected animals, after 10 days). Reference is made to other similar tests to examine the effect of variations in the composition of the basal diet on the response to ACTH.

The Influence of pH Value and of Carbon Source on the Nutritional Requirements of *Acetobacter suboxydans*. By A. N. Hall, K. S. Tiwari and T. K. Walker. (*Faculty of Technology, University of Manchester*)

In the course of a more extended investigation of the nutrition of *Acetobacter* species, results were obtained for *A. suboxydans* A.T.C.C. 621, which presented certain differences from those which Stokes & Larsen (1945) and Underkofler, Bantz & Peterson (1943) obtained with this species.

In attempting to trace the origin of these discrepancies the effects of varying the carbon source and pH of the medium on growth requirements have been studied. The synthetic medium used was a modification of that described by Dunn, Shankman, Camien & Block (1947). Individual amino-acids and vitamins were omitted from the medium and growth was estimated in the Spekker. A decrease to one-fourth or less of the growth in the complete medium was considered to indicate the omission of an essential nutrient, whereas a fall to two-thirds or less was regarded as showing the absence of a stimulatory factor.

In media containing glycerol or sorbitol, *A. suboxydans* is more exacting at pH 5·8 than at pH 4·6. In glucose media, however, the requirements are most complex at the lower pH value. The range pH 5·0–5·3 appears to be critical in that in glucose media the alanine requirement changes from essential to stimulatory, and in glycerol media the valine requirement changes from non-essential to essential. The fact that valine is not required by the organism in glucose media, whereas it is required in glycerol media at pH 5·3 or higher, points to a function of glucose in valine synthesis not possessed by glycerol.

Similar experiments have been directed towards the vitamin requirements of the organism in the same medium. Nicotinic acid has been found to be an essential factor in glycerol media at pH 5·0 and 6·0. However, in the presence of glucose at pH 6·0 the vitamin is not essential, whereas it is essential at pH 5·0.

It is of interest that Doede (1945) has reported that nicotinic acid is essential for *Shigella paradysenteriae* at higher pH values, but not at lower values. These results appear to be the reverse of our findings for *A. suboxydans*.

REFERENCES

Doede, D. R. (1945). *Yale J. Biol. Med.* **17**, 595.
Dunn, M. S., Shankman, S., Camien, M. N. & Block, H. (1947). *J. biol. Chem.* **168**, 1.
Stokes, J. L. & Larsen, A. (1945). *J. Bact.* **49**, 495.
Underkofler, L. A., Bantz, A. C. & Peterson, W. H. (1943). *J. Bact.* **45**, 183.

The Fluorescence of Teeth. By R. L. Hartles and A. G. Leaver. (*Biochemistry Department, School of Dental Surgery, University of Liverpool*)

Stübel (1911) found that when irradiated with ultraviolet light the teeth exhibited an intense blue fluorescence second only in intensity to that of the lens of the eye. Benedict (1928) noted that dentine fluoresced more than enamel and with a bluer light. He also reported that the white spot in enamel indicative of dental caries did not fluoresce and that ashing or boiling with 50% NaOH destroyed the fluorescence. Carious enamel and dentine lose their fluorescence. Tiede & Chomse (1934) believe that the fluorescence is due to organic material, whereas Glassa & Fonda (1938) believe it to be associated with the mineral phase. The nature of the fluorescing materials may be of fundamental importance in studying the aetiology of dental caries. If the active materials prove to be organic it suggests that disintegration of the matrix occurs at a very early stage in the carious process.

On refluxing powdered dentine (containing cementum) with ethylene glycol containing 3% KOH, we obtained a strongly fluorescent extract. The fluorescent materials were concentrated by adsorption on charcoal and subsequent elution. Paper chromatography showed that there was more than one fluorescent substance present in the eluate. Acid hydrolysis of the eluate did not destroy the fluorescence; acetylation and methylation of this hydrolysate produced ether-soluble fluorescing materials. The activity is much stronger than can be accounted for by the amino-acids present.

It is concluded, therefore, that the fluorescence of dentine and cementum is due (at least in part) to organic compounds other than amino-acids.

REFERENCES

Benedict, H. C. (1928). *Science*, **67**, 442.
Glassa, J. & Fonda, G. R. (1938). *J. Amer. chem. Soc.* **60**, 722.
Stübel, H. (1911). *Pflüg. Arch. ges. Physiol.* **142**, 1.
Tiede, E. & Chomse, H. (1934). *Ber. dtsch. chem. Ges.* **676**, 1988 (cited in *Chem. Abstr.* (1935), **29**, 1473).

Chromatographic Examination of Cod-Liver Oil. By R. A. MORTON. (*Department of Biochemistry, University of Liverpool*)

The validity of correction procedures for estimating irrelevant absorption in the determination of vitamin A may be tested by examining the ultraviolet absorption curves of fractions obtained after chromatography on slightly weakened alumina, using light petroleum for development. The earliest fractions to pass through the column show minimal irrelevant absorption in the region 260–290 mμ., but this increases with continued elution. The absorption curve from 310–410 mμ. is constant in form (on a scale where $E_{max.} = 1{\cdot}0$) and is significantly greater from 340–410 mμ. than for *all-trans*-vitamin A acetate.

Later fractions, devoid of vitamin A, show absorption maxima for conjugated di-ene (230–240 mμ.), tri-ene (270 mμ.) and tetra-ene (305 and 320 mμ.) acidic groupings in glycerides. Such absorbing materials are strongly held on the adsorbent, but are eluted by means of ether-light petroleum mixtures and finally by diethyl ether. They do not appear to be artifacts, although acids recovered from the soaps (after saponification, extraction of unsaponifiable matter and subsequent acidification) tend to absorb more strongly as a result of isomerization of non-conjugated polyethenoid acids. The absorption curve of the unsaponifiable fraction has the same form as the vitamin A ester fractions after chromatography. The difference between the observed curves and that of *all-trans*-vitamin A acetate is due to the presence of vitamin A_2 and isomeric vitamin A (e.g. neovitamin A) in variable amounts. Anhydrovitamin A is not a normal contaminant.

The $SbCl_3$ colour test (on the unsaponifiable matter) gives values which in terms of the properties of *all-trans*-vitamin A acetate indicates a lower vitamin A content than corresponds with the gross E value (on the unsap.). Although the results of the colour test support the idea that unsaponifiable extracts exhibit 'linear' irrelevant absorption in the region 310–340 mμ., this is not fully established.

Low potency fish-liver oils (1500 i.u./g.) present special problems not brought out in the examination of rich oils and concentrates. The main clues are the properties of the glycerides and the higher absorption values on the long-wave side of the absorption curve.

The Distribution of Vitamins A_1 and A_2 in Amphibia. By R. M. LOVE, F. D. COLLINS and R. A. MORTON. (*Department of Biochemistry, University of Liverpool*)

The distribution of vitamins A_1 and A_2 has been much studied. Wald (1946) came to the conclusion that fish which spawn in fresh water contain predominantly vitamin A_2 while those that spawn in salt water contain mostly vitamin A_1. There are many apparent exceptions to this and in fact a very large number of fish-liver oils contain a small proportion of vitamin A_2. Vitamin A_2 has also been found in two Amphibia, a newt (*Triturus viridescens*) (Wald, 1942) and the tadpole of the bullfrog (*Rana catesbiana*) (Wald, 1946). The adult bullfrog, however, had only vitamin A_1.

In the present work no vitamin A_2 but only vitamin A_1 has been found in tadpoles and adults of *Rana temporaria* and *R. esculenta*. Even in tadpoles treated with thiourea, which delayed metamorphosis and allowed the animals a longer period in a fresh water environment, no vitamin A_2 was found.

Vitamin A_2 was found in two species of newt (*Triton carnifex* and *T. cristata*). In the former case a detailed study has been made of the absorption spectra of the lipids obtained from the livers, fat bodies, ovaries, testes, spleens, guts and the remainder of the body. The liver contained 1·5 mg. of vitamin A_1 and 2·1 mg. of vitamin A_2 per 100 g. of tissue.

It was thought that an amphibian, the axolotl (*Amblystoma tigrinum*), which was entirely aquatic and which remained in the larval form throughout its life would offer a significant comparison with the aquatic bullfrog tadpole and the partially aquatic newt. Axolotl eggs were hatched and the young axolotls reared in the laboratory. At first they were fed *Daphnia pulex* and a water plant (*Elodea canadensis*). After 4 months they were fed chopped meat or earthworms. No vitamin A could be detected in the livers, but the animals appeared healthy. When vitamin A was given it was stored in the liver: when β-carotene was given there was no conversion to vitamin A and little liver storage of unchanged carotene.

REFERENCES

Wald, G. (1942). *Biol. Symp.* **7**, 43.

Wald, G. (1946). *Harvey Lectures*, **117**.

The Distribution of Vitamin A in the Rat-Liver Cytoplasm. By F. D. COLLINS. (*Department of Biochemistry, University of Liverpool*)

Goerner (1938) reported the presence of vitamin A in mitochondria. This has now been confirmed. A rat liver was separated into four fractions by differential centrifugation in isotonic sucrose by the procedure of Hogeboom, Schneider & Pallade (1948). The nuclear fraction contained 7·0 μg. of vitamin A. This fraction was probably contaminated with intact cells and some mitochondria (cf. Potter, Recknagel & Hurlbert, 1951). The mitochondria contained 41·0 μg. of vitamin A while the microsomes had 0·8 μg. As a Waring blender had been used, the microsomes may have been contaminated with disintegrated mitochondria (cf. Potter *et al.* 1951). Finally the soluble fraction had 156 μg. of vitamin A.

Ernster, Zetterström & Lindberg (1950) have claimed that a water-soluble form of vitamin A can be extracted with trichloroacetic acid solution from liver. An attempt to confirm this was made, but no clear-cut distinction could be made between a water-soluble form of vitamin A and a colloidal solution of lipid droplets containing vitamin A.

REFERENCES

Ernster, L., Zetterström, R. & Lindberg, O. (1950). *Exp. Cell Res.* **1**, 494.
Goerner, A. (1938). *J. biol. Chem.* **122**, 529,
Hogeboom, G. H., Schneider, W C. & Pallade, G. E. (1948). *J. biol. Chem.* **172**, 619.
Potter, V. R., Recknagel, R. O. & Hurlbert, R. B. (1951). *Fed. Proc.* **10**, 646.

DEMONSTRATION

The Fluorescence of Teeth and Tooth Extracts. By R. L. HARTLES and A. G. LEAVER. (*Biochemistry Department, School of Dental Surgery, University of Liverpool*)

[*The Editors of the Biochemical Journal accept no responsibility for the Reports of the Proceedings of the Society. Abstracts of papers read are published as received from the authors.*]

PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **308th Meeting** *of the Biochemical Society was held in the Physiology Institute, Newport Road, Cardiff, on Friday, 23 May* 1952, *when the following papers were read:*

COMMUNICATIONS

The Uptake of ^{35}S from L-Cystine into Brain Tissue. By M. K. GAITONDE and D. RICHTER. (*Neuropsychiatric Research Centre, Whitchurch Hospital, Cardiff*)

L-Cystine labelled with ^{35}S was added to a glucose-phosphate-Ringer medium containing rat brain slices or homogenates. The labelled L-cystine was prepared from yeast by the method of Williams & Dawson (1951, 1952). After incubation in Warburg vessels at 37° for 3 hr. in the presence of 5% CO_2–95% O_2, trichloroacetic acid solution was added and fractions were separated containing (*a*) acid-soluble substances, (*b*) lipids extracted by boiling ethanol-chloroform mixture (1:1), and (*c*) proteins. The total sulphur content of the fractions was determined by oxidation with a mixture of perchloric and nitric acids (Pirie, 1932) and subsequent precipitation as benzidine sulphate. The method was checked by comparison with the Carius method, which gave good agreement.

Radioactive assays made on benzidine sulphate prepared from the three fractions showed that there was a greater incorporation of labelled cystine in the protein fraction than in the lipid fraction. However, about 60% of the activity in the protein fraction was lost on repeated treatment with thioglycollic acid, which indicated that this part was due to the combination of cystine with the protein by the formation of S—S linkages. The remaining activity might be attributed to protein synthesis.

A small fraction of the radioactivity in lipid extracts was attributable to lipoprotein, the protein moiety of which was separated by evaporating the lipid extract to dryness and resuspending in the same solvent (chloroform-ethanol); the major portion of the activity remained in the soluble lipid fraction.

REFERENCES

Pirie, N. W. (1932). *Biochem. J.* **26**, 2044.
Williams, R. B. & Dawson, R. M. C. (1951). *Biochem. J.* **50**, xxi.
Williams, R. B. & Dawson, R. M. C. (1952). *Biochem. J.* (in the Press).

A Combustion Technique for the Assay of Tritium, ^{13}C and ^{14}C in a single 10 mg. sample of Biological Material. By R. F. GLASCOCK (introduced by S. J. FOLLEY). (*National Institute for Research in Dairying, University of Reading*)

It is inconvenient if less than about 75 mg. is available for the solid counting at infinite thickness of ^{14}C-labelled metabolites, and frequently this amount cannot be obtained, e.g. from a tissue slice experiment. On the other hand the quantitative combustion of 10 mg. samples of most organic compounds will yield sufficient carbon dioxide to count by the method of Brown & Miller (1947) and sufficient water for the determination of tritium by the method of Glascock (1951*a*, *b*). By adjusting the activity of the ^{14}C used in the experiment and the size of the $^{14}CO_2$ samples counted, it is possible to set aside sufficient for the mass spectrometric assay of ^{13}C.

A method for the quantitative combustion of 10 mg. samples has been used by the author for the past 3 years for the assay of ^{14}C by gas counting, but as it is applicable without modification to the determination of tritium, ^{13}C and ^{14}C in the same 10 mg. sample, it is thought to have acquired a greater interest in view of the increasing use of these three isotopes simultaneously. The method is therefore described and results presented demonstrating (1) the quantitative combustion of some standard substances, and (2) the closely agreeing results of combusting and assaying in triplicate a sample containing all three isotopes. The advantages of gas counting are also pointed out with special reference

to some results obtained from the counting at infinite thickness of solid samples and the gas counting of carbon dioxide obtained from them on combustion. The conclusions are drawn that gas counting is more efficient by a factor of about 10 permitting a corresponding tenfold reduction in the amount of ^{14}C used, that reduction of all organic compounds to the same compound (either $BaCO_3$ or CO_2) is preferable for the accurate comparison of activities and, since this requires a combustion, that it is easier to count the carbon dioxide than to absorb it in baryta and count the precipitated barium carbonate.

REFERENCES

Brown, S. C. & Miller, W. W. (1947). *Rev. sci. Instrum.* **18**, 496.

Glascock, R. F. (1951*a*). *Nature, Lond.*, **168**, 121.

Glascock, R. F. (1951*b*). *Nucleonics*, **9**, no. 5, 28.

Biological Fractionation of Hydrogen Isotopes in Mammary Gland and other Tissues. By R. F. Glascock and W. G. Duncombe (introduced by S. J. Folley). (*National Institute for Research in Dairying, University of Reading*)

The differences in biological behaviour between light and heavy hydrogen compounds, especially water, are well known and have been ably summarized by Thorn (1951). Although concentrations of D_2O as low as 10% are known to affect the rate of biochemical processes biological fractionation of light and heavy hydrogen has never been satisfactorily demonstrated. Thorn suggests that variations in the amount of deuterium in biological systems may be of the order of 3%, but it is obvious that variations much larger than this could occur in nature and still be undetected because of the low natural abundance of deuterium (0·02 atom %) and the difficulty in measuring it accurately.

It is usually assumed, however, that at concentrations of about 3 atoms % deuterium is a valid label for hydrogen which implies that both isotopes are incorporated into biological products in the same ratio as they occur in the substrate water. The D/H mass ratio is 2 to 1, and if the above assumption is valid then the parallel assumption that the uptake of tritium from doubly labelled water is relatively the same as that of deuterium should also be valid since the T/D mass ratio is only 1·5 to 1. With the development of a satisfactory routine method for the determination of tritium (Glascock, 1951 *a*, *b*) it became possible to test this assumption. The body water of lactating nursing rats was labelled with the two isotopes to a level of about 2·5% D_2O and 1 μc. per ml. TOH. This isotope level was maintained over 2 weeks by supplying drinking water labelled with the isotopes in the same ratio.

At the end of this period mothers and pups were killed and the T/D ratio in fatty acids from various organs determined. Taking the T/D ratio as unity in the drinking water it was found to be markedly lower in the fatty acids from most sources especially mammary gland where it was 0·8. This same ratio was found in the body fat of the pups. Maternal body fat was too poor in isotopes for deuterium to be determined accurately, but maternal liver fat from some animals was relatively richer in tritium than was mammary gland fat.

These results indicate that the incorporation of tritium into fatty acids may be less than that of deuterium by as much as 20% and it seems reasonable to deduce that the difference between deuterium and hydrogen uptake is of the same order. It is to be noted that the deuterium concentration used was at the low level (2·5%) usually assumed suitable for biological work and that 1 μc./ml. of tritium corresponds with only about 4×10^{-8} atoms %.

REFERENCES

Glascock, R. F. (1951*a*). *Nature, Lond.*, **168**, 121.

Glascock, R. F. (1951*b*). *Nucleonics*, **9**, no. 5, 28.

Thorn, M. B. (1951). *Biochem. J.* **49**, 602.

Metabolic Control by Intracellular pH. By P. C. CALDWELL and E. J. HARRIS. (*Biophysics Department, University College, London*)

For many types of cell there is good evidence that the ratio (internal H^+ concentration)/external H^+ concentration) corresponds closely to that calculated from the Donnan equation (see, for instance, Boyle & Conway, 1941). This can be written in the form

$$\frac{(H^+ \text{ internal})}{(H^+ \text{ external})} = \exp(EF/RT), \qquad (1)$$

where E is the potential difference across the membrane, the outside of the cell being taken as positive.

In the case of frog muscle in a medium containing 2 millimolar K^+, E is about 90 mV., so that if (H^+ external) is 6×10^{-8} M (pH 7·20), (H^+ internal) will be 2×10^{-6} M (pH 5·6). This high internal H^+ concentration can be reduced by any factor which leads to a diminution of membrane potential. These factors include (*a*) oxygen deficiency, (*b*) electrical stimulation and (*c*) increased external K^+ concentration and it is noteworthy that they also bring about changes in metabolism. Thus (*a*) brain slices consume glucose more rapidly under anaerobic conditions than aerobically (Dixon, 1936), (*b*) electrical stimulation of brain tissue, nerve or muscle leads to an increased oxygen use or heat production (Hill, 1949; McIlwain, 1951), and (*c*) increase of external K^+ causes an increased heat production in muscle (Solandt, 1936), an acceleration of glycogen synthesis by liver slices (Buchanan, Hastings & Nesbett, 1949) and higher oxygen consumption and lactic acid formation by brain slices (Ashford & Dixon, 1935).

Consideration of the pH-activity curves of both homogenates and of isolated enzymes suggests that the acceleration of glycolytic and other reactions brought about by depolarizing conditions when undamaged cells are used may arise from the reduction of internal H^+ concentration causing an increased enzyme activity. This interpretation provides an explanation of many aspects of the Pasteur effect, in particular its dependence upon the cell structure being intact.

REFERENCES

Ashford, C. A. & Dixon, K. C. (1935). *Biochem. J.* **29**, 157.
Boyle, P. J. & Conway, E. J. (1941). *J. Physiol.* **100**, 1.
Buchanan, J. M., Hastings, A. B. & Nesbett, F. B. (1949). *J. biol. Chem.* **180**, 435.
Dixon, K. C. (1936). *Biochem. J.* **30**, 1479.
Hill, A. V. (1949). *Proc. roy. Soc.* B, **136**, 195.
McIlwain, H. (1951). *Biochem. J.* **50**, 132.
Solandt, D. Y. (1936). *J. Physiol.* **86**, 162.

A Study of Congenital Porphyria. By J. E. KENCH and C. S. PAPASTAMATIO. (*Department of Haematology, Manchester Royal Infirmary and University*)

A study was initiated in May 1947 on a case of congenital porphyria already described (Ashby, 1926). This female patient, aged 25 years, had remained in fairly good health since childhood, but showed signs of increased breakdown of red blood cells so often encountered in this disease (Aldrich, Hawkinson, Grinstein & Watson, 1951), namely, reticulocytosis, nucleated red blood cells, hyperplastic bone marrow, and increased urobilinogen excretion (mean for 22 days, 631 mg./day) (cf. Gray, Muir & Neuberger, 1950). The patient was pregnant, and was delivered normally of a full-term male child on 17 July 1947.

Porphyrins in red blood cells and plasma, urine and faeces of mother and child were studied, as well as breast milk and meconium, quantitatively and subsequently qualitatively by the paper chromatographic procedures of Nicholas & Rimington (1949), and Chu, Green & Chu (1951). The foetal blood at birth contained 256 and 306 μg. porphyrin/100 ml. red blood cells and plasma respectively, the corresponding values in the mother being 544 and 774 μg./100 ml. The new-born child was normal, and no porphyrins could be detected in the blood on the 6th and subsequent post-natal days. The meconium was very dark in colour, and contained 9·65 mg. porphyrin, comprising about equal quantities of 8-, 6-, 4-, 3- and 2-carboxylic porphyrins. Uroporphyrin was observed in the meconium, although it was absent from maternal faeces. The baby's urine contained 140 μg. porphyrin on the 2nd day, but none could be detected in later specimens. From these findings, at variance with earlier experiments (Hammer, 1930), it is apparent that the placenta is permeable to porphyrins, but the latter are rapidly eliminated by the foetus. No porphyrin was found in the breast milk.

During a second pregnancy, in 1949, the condition of the patient deteriorated and she died in liver failure. Post-mortem examination of the tissues

revealed the presence of many porphyrin intermediates, as described by Nicholas & Rimington (1951) in meconium, urine and faeces. Paper chromatography applied to liver separated discrete fluorescent spots corresponding to porphyrins with 8-, 7-, 6-, 4-, 3- and 2-carboxyl groups, whilst the spleen contained the complete series of 2- to 8-carboxylic porphyrins.

REFERENCES

Aldrich, R. A., Hawkinson, V., Grinstein, M. & Watson, C. J. (1951). *Blood*, **6**, 685.
Ashby, H. T. (1926). *Quart. J. Med.* **19**, 375.
Chu, T. C., Green, A. A. & Chu, E. J. (1951). *J. biol. Chem.* **190**, 643.
Gray, C. H., Muir, I. M. H. & Neuberger, A. (1950). *Biochem. J.* **47**, 542.
Hammer, H. (1930). *Virchows Arch.* **277**, 159.
Nicholas, R. E. H. & Rimington, C. (1949). *Scand. J. clin. lab. Invest.* **1**, 12.
Nicholas, R. E. H. & Rimington, C. (1951). *Biochem. J.* **48**, 306.

Vitamin D Balance Experiments in Rats given a Massive Dose of Calciferol. By E. M. Cruickshank and E. Kodicek. (*Dunn Nutritional Laboratory, University of Cambridge and Medical Research Council*)

Since an accurate appraisal of the fate of ingested vitamin D is not possible from the scanty data available (Vollmer, 1939; Morgan & Shimotori, 1943; Houet, 1946), balance studies on rats were undertaken to gain further information. Weanling rachitic rats received *per os* 1 mg. calciferol (40 000 i.u.) in arachis oil and on each of the four succeeding days the livers, intestines (minus contents), the remainder of the carcasses and the faeces of five rats were freeze-dried and kept at $-20°$ until assayed. Tissues from another group which received only oil were also tested. The dried samples were assayed radiographically during the next 18 months. Replicate assays during this period showed that no loss of the vitamin occurred during storage. That no detectable loss resulted from freeze-drying was indicated by assays on fresh material.

In the undosed rats, little or no vitamin D was detected. In the rats receiving calciferol, the total vitamin content of the body in i.u. was 2380, 1230, 1260 and 940 on the four consecutive days. The fall on the second day after dosing was mainly due to a decrease in the liver from the initial value of 1440 to 510, a level subsequently maintained. The intestines showed a steady decrease from 250 on the first day to 40 i.u. on the fourth day. The remainder of the carcass maintained a level of about 600 i.u. throughout. It thus appears that the liver is the first main site of deposition. Within the period studied no significant shift of the vitamin from liver to carcass was observed.

The vitamin D excretion in the faeces decreased rapidly, the average total amount in i.u. on the four consecutive days being 4860, 1380, 200 and 30. No vitamin D was found in urine.

From the results of all experiments, the vitamin D in the body constituted about 6 %, and in the faeces on the first two days, 17 % of the dose. The fate of the rest of the ingested vitamin is not known. It is assumed that it is converted, possibly in the gut or in the faeces after excretion, into inactive degradation products.

We are most grateful to Mr P. Armitage for the statistical analyses and to Dr R. Gane for the facilities provided at the Low Temperature Station.

REFERENCES

Houet, R. (1946). *C.R. Soc. Biol., Paris*, **140**, 1029.
Morgan, A. F. & Shimotori, N. (1943). *J. biol. Chem.* **147**, 189.
Vollmer, H. (1939). *Amer. J. Dis. Child.* **57**, 343.

Arylsulphatase Activity in Marine Molluscs. By K. S. Dodgson, J. I. M. Lewis and B. Spencer. (*Physiology Institute, Newport Road, Cardiff*)

Arylsulphatase is known to occur in certain marine molluscs of tropical origin (Soda, 1940), but no studies on comparable temperate organisms appear to have been made. We have applied the *p*-acetylphenylsulphate method (Dodgson, Spencer & Thomas, 1952) for the estimation of arylsulphatase activity to a number of marine gastropods common to the British Isles and have found them to be rich sources of the enzyme. Amongst the Prosobranchiata activity was considerable in the digestive organs of both herbivores and carnivores, and the common limpet (*Patella vulgata*) and the large peri-

winkle (*Littorina littorea*) were selected for further study.

Homogenates of the visceral humps of both these organisms showed greatest activity at pH 5·5 in 0·5M-acetate buffer, but whereas the optimum substrate concentration of *Patella* was 0·065M that of *Littorina* was 0·04M. When assayed under these conditions the arylsulphatase activity of both organisms varied between 7000 and 140 000 units/g. (1 unit = 1 μg. *p*-hydroxyacetophenone released in 1 hr. at 37·5° under the optimal conditions), with the values tending towards the higher level during starvation. In the case of *Littorina* particularly it is doubtful whether the increase during starvation can be attributed to loss of body weight only. Further investigation of this increase and of the effect of certain inorganic ions normally present in high concentrations in the tissues is being made with the view to establishing standard conditions for enzyme assay.

It is interesting to compare the optimum conditions for mollusc arylsulphatase action with those established for arylsulphatases of plant and mammalian origin when potassium *p*-acetylphenylsulphate is the substrate. Takadiastase (Parke-Davies and Co., containing added lactose) is optimally active at pH 6·2–6·4 in acetate buffer and 0·004M substrate whilst comparable figures for rat liver arylsulphatase are pH 7·2 and 0·007M. Under these conditions the activity of takadiastase is of the order of 1300 units/g. and that of rat liver homogenates (M.R.C. hooded rats) 1500–4500 units/g.

The high concentrations of arylsulphatase existing in the digestive organs of molluscs is accompanied by high β-glucuronidase activity and the properties of this enzyme are now being studied.

We wish to thank Dr F. S. Russell and the staff of the Marine Biological Laboratories, Plymouth, for valuable advice.

REFERENCES

Dodgson, K. S., Spencer, B. & Thomas, J. (1952). *Biochem. J.* **51**, i.
Soda, T. (1940). In Baumann, E. & Myrback, K., *Die Methoden der Fermentforschung*, p. 1695. Leipzig: Springer.

Purification of the Arylsulphatase of *Patella vulgata*. By K. S. Dodgson and B. Spencer.
(*Physiology Institute, Newport Road, Cardiff*)

The high arylsulphatase activity of the limpet (*Patella vulgata*) suggests that this organism might be used for the preparation of arylsulphatase concentrates. Similar concentrates obtained from the digestive glands of *Charonia lampas* (*Triton nodiferus*), a large tropical mollusc, have been reported by Soda & Egami (1941), but it is not known whether separation of the enzyme from β-glucuronidase was achieved. In the present work preliminary autolysis at 37·5° as described by Soda & Egami (1941) was considered inadvisable since buffered homogenates of the viscera of *Patella* rapidly lose arylsulphatase activity at this temperature.

Briefly, the following procedure was adopted: (*a*) preparation of an acetone-dried powder of homogenized limpet viscera, (*b*) fractional precipitation of an aqueous solution of this powder with acetone, (*c*) removal of β-glucuronidase, which is present in high concentrations, by precipitation with proflavin sulphate, and (*d*) precipitation of the arylsulphatase with acetone. The stable light-coloured powder thus obtained (activity 3 000 000 *p*-hydroxyacetophenone units/g., see previous communication) is quite free from β-glucuronidase and represents a 100-fold concentration of the enzyme with relatively little loss of the total activity.

The concentrate is optimally active at pH 5·5 in 0·5M-acetate buffer and at a substrate concentration of 0·055M when potassium *p*-acetylphenylsulphate is the substrate. The influence of certain activators and inhibitors on the activity of the concentrate has revealed striking differences between the arylsulphatases of rat liver, limpet viscera and *Aspergillus oryzae*. Chauncey, Seligman & Nachlas (1951) have reported a 50% activation of rat liver arylsulphatase by $MgCl_2$, but this compound does not affect takadiastase. The 10% activation of the limpet enzyme by 0·01M-$MgCl_2$ can be attributed to Cl' rather than Mg^{++} since NaCl, KCl and $BaCl_2$ (which are without effect on takadiastase) gave similar activation, whereas magnesium acetate was without effect. Dzialoszynski (1950) has observed complete inhibition of the arylsulphatase of Clarase by concentrations of phenylhydrazine (10^{-5}M) which have no effect on the enzymes of rat liver and limpets. Inorganic sulphate is without effect on takadiastase, but in concentrations of 0·01M causes 50% inhibition of the limpet enzyme. Phosphate (0·01M) completely inhibits the limpet concentrate, but at

this molarity the effect on takadiastase and rat liver is slight.

Attempts are being made to obtain purified preparations of the arylsulphatases of rat liver and takadiastase with a view to an extension of these comparative studies.

REFERENCES

Chauncey, H. H., Seligman, A. M. & Nachlas, M. M. (1951). *Stain Tech.* **26**, 19.

Dzialoszynski, L. M. (1950). *Nature, Lond.*, **166**, 157.

Soda, T. & Egami, F. (1941). *J. chem. Soc. Tokyo*, **62**, 256.

The Xanthoproteic Test. By J. A. SAUNDERS. (*Department of Physiology and Biochemistry, Medical School, King's College, Newcastle-upon-Tyne*)

The xanthoproteic test has been investigated by mixing 1 ml. 6·25 mM-salicylate with 1 ml. nitric acid solution, heating by immersion in a boiling water bath and then adding 10% (w/v) NaOH solution until alkaline. It has been found that the nitric acid solution can be varied from 10 to 40% (v/v) and the time of heating from 5 to 10 min. without any variation in the colour produced.

Using pure nitric acid, which has been thoroughly aerated to remove oxides of nitrogen, no colour is produced with 0·3 mM-salicylate, but the addition of traces of sodium nitrite before heating, produces an appreciable colour. This effect is given by 0·4 mM-nitrite in the reaction mixture, but not by smaller concentrations. Some colour can be obtained with 0·3 mM-salicylate by using longer heating times (up to 1 hr.) or by adding conc. nitric acid with 5 min. heating, but neither procedure is as effective as the addition of nitrite.

No colour is produced with either concentration of salicylate when the salicylate solution contains 0·5 mM-hydrogen peroxide or 0·1% (w/v) urea. The effect of urea can be prevented by adding a calculated excess of nitrous acid.

The colour is not altered by the presence of 0·05M ammonium sulphate, guanidine hydrochloride, glycine, alanine, sodium glutamate, arginine or glucose in the salicylate solution.

The test is positive with *p*-hydroxy benzoate, *p*-aminobenzoate, sulphanilic acid, sulphanilamide, tyrosine and tryptophan (2·5 mM solutions). A detectable yellow colour is given by 0·05 mM salicylate, tyrosine or tryptophan (using nitrite). This is the same sensitivity as that reported for Millon's test for tyrosine, but less than that for the glyoxylic acid test for tryptophan (Lloyd & Shore, 1938).

No colour is given by benzoate, phthalate, phenylglycine, phenylalanine, tocopherol or vitamin K (5 mM solutions). Phenylalanine gives no colour when a 0·1M solution is boiled with concentrated nitric acid and made alkaline.

REFERENCE

Lloyd, D. J. & Shore, A. (1938). *Chemistry of the proteins*, 2nd ed., p. 51. London: Churchill.

Chemical Tests in the Study of Thyroid Disease. By A. L. TÁRNOKY. (*Royal Berkshire Hospital, Reading*)

This is a preliminary survey of an investigation in which the following values are determined: the basal metabolic rate (B.M.R.) on two consecutive mornings, pigments and creatinine concentrations in both mid-morning urines, and fasting cholesterol, creatinine and creatine (Griffiths, 1951) levels in serum obtained on the first day.

The first 31 patients of this series (females, age 23–68 years) had B.M.R. −51 to +60% (mean +7, S.D. 26), serum cholesterol 120–608 mg./100 ml. (mean 278, S.D. 92·7), serum creatinine 0·8–2·0 mg./100 ml. (mean 1·17, S.D. 0·30) and serum creatine 0–1·0 mg./100 ml. (mean 0·57, S.D. 0·22). The day's lowest corrected O_2 consumption was 126–316 ml./min. on the first and 118–288 ml./min. on the second day. The mean consumption on the second was 99·0% of the first day (S.D. 12·1). Urinary creatinine concentrations ranged from 13 to 136 mg./100 ml. on the first and 15–131 mg./100 ml. on the second day. The mean second-day concentration was 113·7% of

the first day (S.D. 44·2). Values of the urinary pigment:creatinine ratio U.PG./U.CR. (Vorzimmer, Cohen & Joskow, 1949), expressed as

$$\frac{1000E \text{ of urine (Ilford 601 filter)}}{\text{mg. creatinine/100 ml.}}$$

were 1·8–23·0 and 1·5–22·4 on the 2 days, the average second-day figure being 96·2 % of the first day's (S.D. 22·1). Correlations involving U.PG./U.CR. do not include one patient menstruating at the time.

The rise or fall in the O_2 uptake from one day to the next was significantly correlated (for $P = 0{\cdot}05$) with similar changes in U.PG./U.CR. ($\chi^2 = 3{\cdot}93$) but not with urinary creatinine ($\chi^2 = 3{\cdot}66$). Significant correlation was obtained between the serum cholesterol and serum creatinine levels ($r = 0{\cdot}52$), serum cholesterol and U.PG./U.CR. ($-0{\cdot}64$), B.M.R. and U.PG./U.CR. (0·45) and between B.M.R. and serum cholesterol ($-0{\cdot}54$). This last figure differs from the results of Peeler, Hepler, Kinney, Cisler & Jung (1950) who found $r = -0{\cdot}03$ in normal males. There was no significant correlation between B.M.R. and urinary creatinine ($r = -0{\cdot}20$), B.M.R. and serum creatinine ($-0{\cdot}11$), B.M.R. and serum creatine (0·05), serum cholesterol and creatine ($-0{\cdot}07$), U.PG./U.CR. and serum creatinine ($-0{\cdot}12$) or U.PG./U.CR. and serum creatine (0·17). The regression equation

$$\text{B.M.R.} = -2{\cdot}8 + 2{\cdot}2 \text{ U.PG./U.CR.}$$

is similar to the one given by Vorzimmer *et al.* for women.

REFERENCES

Griffiths, W. J. (1951). *Lancet*, **2**, 467.

Peeler, A. L., Hepler, O. E., Kinney, V. M., Cisler, L. E. & Jung, F. T. (1950). *J. appl. Physiol.* **3**, 197.

Vorzimmer, J. J., Cohen, I. B. & Joskow, J. (1949). *J. Lab. clin. Med.* **34**, 482.

Paper Chromatography of Urinary Phenolic Acids, Polyphenols and Oestrogens. By R. J. BOSCOTT. (*Department of Anatomy, University of Birmingham*)

Boscott (1951) reported the use of aqueous alkali of high salt concentration for the paper chromatographic separation of urinary phenolic acids. Two-dimensional chromatography of these compounds and urinary polyphenols using benzene 2, glacial acetic acid 2 and water 1 (Bray, Lake, Thorpe & White, 1950) as the first solvent system and 20 % (w/v) KCl at room temperature (polyphenol fraction) or at 34° (phenolic acid fraction) gave good resolutions of the complex acidic and polyphenolic fractions obtained from hydrolysed urines. The separated components were located by ultraviolet fluorescence and colour changes on neutral and alkaline paper, spotting with droplets of 90 % H_2SO_4 and finally spraying the same paper with a diazonium reagent (diazotized *p*-*p*^{1}-diaminodiphenylsulphone, sulphanilic acid, dehydrothiotoluidine sulphonic acid, or *p*-nitraniline). Results obtained from urines of rats on two diets will be reported.

The addition of LiCl or $CaCl_2.2H_2O$ to mixtures of toluene and absolute methanol or ethanol caused the mixture to separate into two layers. The lower alcohol layer was sprayed on paper to serve as stationary organic solvent in conjunction with the toluene layer used as the mobile phase, for separation of relatively weakly polar steroids.

Diserens (1948), McKee (1946), Neuberg (1916), Hahn (1932) and Tamba (1924) reported the use of hydrotropic agents to increase the water solubility of vat dyes, nitrobenzene, ethyl acetoacetate, drugs and cholesterol. Hydrotropic agents include urea, thiourea, hexamethylenetetramine, triethanolamine borate, betaines, ammonium thiocyanate, lithium iodide and numerous alkali salts of aromatic and aliphatic acids.

Bentley & Whitehead (1949) used urea as a solute in their water-miscible organic solvents for paper chromatography in order to obtain sharper outlines of the separated components. The use of concentrated solutions of hydrotropic agents such as sodium *p*-toluenesulphonate as stationary phase and water-immiscible organic solvents as mobile phase in the paper chromatographic separation of stereoisomeric oestrogens and androgens will be reported.

REFERENCES

Bentley, H. R. & Whitehead, J. K. (1949). *Nature, Lond.*, **164**, 182.

Boscott, R. J. (1951). *Biochem. J.* **48**, xlviii.

Bray, H. G., Lake, H. J., Thorpe, W. V. & White, K. (1950). *Biochem. J.* **47**, xiii.

Diserens, L. (1948). *The Chemical Technology of Dyeing and Printing* (translated from the Second German Edition). New York: Reinhold Publishing Corporation, New York, U.S.A.

Hahn, F. von (1932). *Kolloidzschr.* **62**, 202.

McKee, R. H. (1946). *Industr. Engng Chem.* **38**, 382.

Neuberg, C. (1916). *Biochem. Z.* **76**, 107.

Tamba, R. (1924). *Biochem. Z.* **145**, 415.

Effect of Nitrogen Mustard or X- or Radium-Irradiation Treatment on the Level of Blood Serum Protein-bound Carbohydrate (Polysaccharide) in Patients with Neoplasms. By J. W. KEYSER. (*Department of Pathology and Bacteriology, Welsh National School of Medicine, Cardiff*)

Increases in the level of serum polysaccharide in various diseases have been reported by many investigators. These increases are attributed by some to tissue destruction (Seibert, Seibert, Atno & Campbell, 1946), and by others to tissue proliferation or repair (Shetlar, Bryan, Foster, Shetlar & Everett, 1949). The present investigation was undertaken to find out (1) what effect nitrogen mustard or irradiation treatment might have on the level of serum polysaccharide in patients with various neoplastic diseases, in whom raised values are usually found, and (2) how much of the increase in polysaccharide is due to the mucoprotein fraction (Winzler, Devor, Mehl & Smyth, 1948). Fourteen cases were studied, polysaccharide being estimated by the orcinol method (Sørensen & Haugaard, 1933; Rimington, 1940). It was found that, in general, treatment that resulted in marked clinical improvement and reduction in the size of the tumour was associated with a fall in the level of serum polysaccharide (total and mucoprotein) towards normal values, but a preliminary rise during treatment was often observed; and with relapse the polysaccharide level rose again. The mucoprotein polysaccharide rose and fell with the total but did not constitute a constant proportion of it. In most cases serial white blood cell counts were made, and in a few cases it was practicable to measure the excretion of nitrogenous substances in the urine during treatment. Our findings do not appear to support decisively either the 'tissue breakdown' or the 'tissue proliferation' hypothesis. Possibly these are reconcilable in the light of the work of Gersh and Catchpole (Catchpole, 1950; Gersh & Catchpole, 1949), which suggests that a process of depolymerization takes place in the tissue around tumours, whereby water-soluble glycoproteins are produced.

I am grateful to Prof. J. Gough, who suggested the investigation, for his help, and to the clinicians and many others who co-operated.

REFERENCES

Catchpole, H. R. (1950). *Proc. Soc. exp. Biol., N.Y.*, **75**, 221.
Gersh, I. & Catchpole, H. R. (1949). *Amer. J. Anat.* **85**, 457.
Rimington, C. (1940). *Biochem. J.* **34**, 931.
Seibert, F. B., Seibert, M. V., Atno, A. J. & Campbell, H. W. (1946). *J. Clin. Invest.* **26**, 90.
Shetlar, M. R., Bryan, R. S., Foster, J. V., Shetlar, C. L. & Everett, M. R. (1949). *Proc. Soc. exp. Biol., N.Y.*, **72**, 294.
Sørensen, M. & Haugaard, G. (1933). *Biochem. Z.* **260**, 247.
Winzler, R. J., Devor, A. W., Mehl, J. W. & Smyth, I. M. (1948). *J. clin. Invest.* **27**, 609.

A Note on the Serum Tryptophan-Perchloric Acid Reaction. By J. W. KEYSER. (*Department of Pathology and Bacteriology, Welsh National School of Medicine, Cardiff*)

The tryptophan-perchloric acid (TA) reaction of Cohen (1944) was applied to blood serum by Seibert, Pfaff & Seibert (1948) and has been generally assumed to measure the serum polysaccharide content. In the present study, analysis of 110 specimens of serum from about 60 people (including normals, patients with neoplastic diseases, and accident cases) showed significant correlation between the strength of the TA reaction and the total protein-bound hexose as estimated with orcinol (Sørensen & Haugaard, 1933; Rimington, 1940). Serum from a very jaundiced patient, however, gave a TA value much above that corresponding to its polysaccharide content. These observations agree with the findings of Shetlar, Foster, Kelly, Shetlar, Bryan & Everett (1949). Fluctuations in the concentration of total protein-bound hexose, e.g. during and after nitrogen mustard therapy (Keyser, 1952*a*) and after burning (Keyser, 1952*b*), were reflected in the strength of the TA reaction. The reacting substance is precipitated by ethanol and by perchloric acid with the proteins. Serum from a case of advanced pulmonary tuberculosis dialysed for 18 hr. against running tap water (Visking 'NoJax' cellulose casing) lost only about 8 or 9% of its TA-reacting material. A normal serum also lost 8–9%. Spectrophotometric study of the TA reaction mixtures obtained with serum from normal people and from burn and cancer cases shows that these give the same type of curves, and a similar curve is given by the ethanol-precipitated total serum proteins. Impure specimens of polysaccharide prepared from ox blood serum as described

by Rimington (1931) gave a strong reaction, as did a specimen of ox serum mucoid kindly supplied by Prof. Rimington. However, the absorption curves obtained with these preparations differed somewhat from those obtained with serum. Specific extinction coefficients, representing the strength of the TA reaction calculated to 1 mg. of hexose, have been determined for a number of protein fractions as well as for the ox serum polysaccharide.

Perchloric acid (60%) giving unsatisfactory colour development (Keyser, 1950) can be rendered suitable for the test by prolonged boiling—an operation that must be carried out with precautions against the risk of explosion.

REFERENCES

Cohen, S. S. (1944). *J. biol. Chem.* **156**, 691.
Keyser, J. W. (1950). *J. clin. Path.* **3**, 106.
Keyser, J. W. (1952*a*). Preceding abstract.
Keyser, J. W. (1952*b*). *J. clin. Path.* **5**, 194.
Rimington, C. (1931). *Biochem. J.* **25**, 1062.
Rimington, C. (1940). *Biochem. J.* **34**, 931.
Seibert, F. B., Pfaff, M. L. & Seibert, M. V. (1948). *Arch. Biochem.* **18**, 279.
Shetlar, M. R., Foster, J. V., Kelly, K. H., Shetlar, C. L., Bryan, R. S. & Everett, M. R. (1949). *Cancer Res.* **9**, 515.
Sørensen, M. & Haugaard, G. (1933). *Biochem. Z.* **260**, 247.

DEMONSTRATIONS

Serial Production of Glass Homogenizers. By D. H. Brown, K. S. Dodgson, T. J. Sherwood and B. Spencer. (*Physiology Institute, Newport Road, Cardiff*)

The manufacture of glass homogenizers as described by Potter & Elvehjem (1936) is not easily adapted to serial production and the resultant components are unstandardized and non-interchangeable. A more convenient method of production requiring the use of a lathe has been developed in this laboratory.

A pouring lip is moulded at one end of a 20 cm. length of Veridia standard bore glass tubing (Chance Bros.) of internal diameter x cm. The inside surface of the tube is ground by moving it backwards and forwards along a lead lap (length 20 cm., diameter $x-0{\cdot}0075$ cm.) which is rotating in a lathe at medium speed. Carborundum powder is used as the grinding agent and there is relatively little wear of the lap when water is the lubricant. The tube (internal diameter now $x+0{\cdot}0025$ cm.) is cleaned with HNO_3 and sealed at the unlipped end, the bottom being hemispherically shaped with a carbon former.

To make the pestle, quill tubing (22 cm. long and of suitable diameter) is joined to a 8 cm. length of thick-walled tubing (diameter $\simeq x$ cm.) which is then sealed off. The bulb of the pestle is blown to shape in a cylindrical mould with a hemispherical end (internal length 5 cm., diameter $x+0{\cdot}020$ cm.). After sealing small teeth on to the bottom of the pestle the shaft is held in the chuck of the lathe and the head accurately aligned.

Hollow cylindrical lead laps (internal diameter $x+0{\cdot}030$ cm.) are used to grind the pestle until the diameter of the head is $x-0{\cdot}0075$ cm. at which stage all irregularities have been removed and the ground surface covers the whole of the cylindrical portion of the head. Finally the teeth of the pestle are ground in a hemispherical mould until they are flat-topped and about 1 mm. in length.

Pestles and tubes made in this way are fully interchangeable. The precision of the parallel cylindrical surfaces and the hemispherical bottoms of the tube and pestle, together with the accurate alinement of the shaft and head of the pestle, increase the efficiency and ease of operation of the homogenizers and reduce breakages to a minimum. When in use pestles are belt driven from a 1/50th h.p. electric motor. The slackness of the belt allows for sudden stoppage of the pestle without fracture.

REFERENCE

Potter, V. R. & Elvehjem, C. A. (1936). *J. biol. Chem.* **114**, 495.

The Cup-Plate Assay of Enzymes and its Application to the Study of Micro-Fungi. By J. Dingle, W. W. Reid and G. L. Solomons. (*Research Department, H. W. Carter and Co. Ltd., The Royal Forest Factory, Coleford, Glos.*)

FORTHCOMING PAPERS

It is hoped to publish the following papers in the next issue of the *Biochemical Journal*:

Nomenclature of amino-acids and vitamins

The relation between pyridoxin and tryptophan metabolism, studied in the rat. By C. E. Dalgliesh

The virulence-enhancing factor of mucins:

5. The different components of the 'third factor' involved in virulence enhancement. By H. Smith, R. C. Gallop and J. L. Stanley

6. Identification of a heparin as the main component of the third factor involved in virulence enhancement By H. Smith, R. C. Gallop, Patricia W. Harris-Smith and J. L. Stanley

Addendum—Physico-chemical examination of polysaccharide A. By J. M. Creeth and B. R. Record

The oxidation of nicotinic acid by *Pseudomonas fluorescens*. By A. Pinsky and M. Michaelis

o-Phenylenediamine and 1:2-diamino-4-nitrobenzene as reagents for α-keto acids. By D. J. D. Hockenhull and G. D. Floodgate

The metabolism of succinic acid in the rumen of the sheep. By A. Kaars Sijpesteijn and S. R. Elsden

Studies on cholinesterase. 8. Determination of reaction velocity constants with a reversible inhibitor of pseudo-cholinesterase. By D. K. Myers

Volatile acids in the secretion products (hair grease) of the skin. By E. Brouwer and H. J. Nijkamp

The occurrence of growth factors for *Lactobacillus leichmannii*, *Streptococcus faecalis* and *Leuconostoc citrovorum* in the tissues of pernicious anaemia patients and controls. By R. H. Girdwood

Some observations on the hydrolysis and extraction of formaldehydogenic corticosteroids. By R. I. S. Bayliss

The content of glycogenolytic factor in some insulin samples. By Genevieve Audy and Margaret Kerly

Appendix—Statistical examination of multiple-dose assays. By N. W. Please

The content of glycogenolytic factor in pancreas from different species. By Genevieve Audy and Margaret Kerly

The effect of varying amounts of dietary cholesterol and of choline upon liver lipids. By Jessie H. Ridout, C. C. Lucas, Jean M. Patterson and C. H. Best

The analysis of hair keratin. 1. Application of microbiological techniques to hydrolysates of human hair. By Jessie M. Lang and C. C. Lucas

Further isotopic studies on haemoglobin formation in the rat and rabbit. By Helen M. Muir, A. Neuberger and J. C. Perrone

Lignin in young plants. By Hanan Meyer and A. Bondi

The estimation of serum bilirubin. By J. Patterson, J. Swale and Cora Maggs

Lipovitellin. 2. The influence of water on the stability of lipovitellin and the effects of freezing and of drying. By C. H. Lea and J. C. Hawke

The effects and the fate of malononitrile and related compounds in animal tissues. By J. Stern, H. Weil-Malherbe and R. H. Green

The oxidation of manganese by enzyme systems. By R. H. Kenten and P. J. G. Mann

Hydrogen peroxide formation in oxidations catalysed by plant α-hydroxyacid oxidase. By R. H. Kenten and P. J. G. Mann

The biochemistry of the nitrifying organisms:

1. The ammonia-oxidizing systems of *Nitrosomonas*. By H. Lees

2. The free-energy efficiency of *Nitrosomonas*. By T. Hofman and H. Lees

The ultraviolet absorption spectrum of ribonuclease. By D. Shugar

Further observations on the preparation and composition of the hyaluronic acid complex of ox synovial fluid. By A. G. Ogston and J. E. Stanier

Separation of adenosine phosphates by paper chromatography and the equilibrium constant of the myokinase system. By L. V. Eggleston and R. Hems